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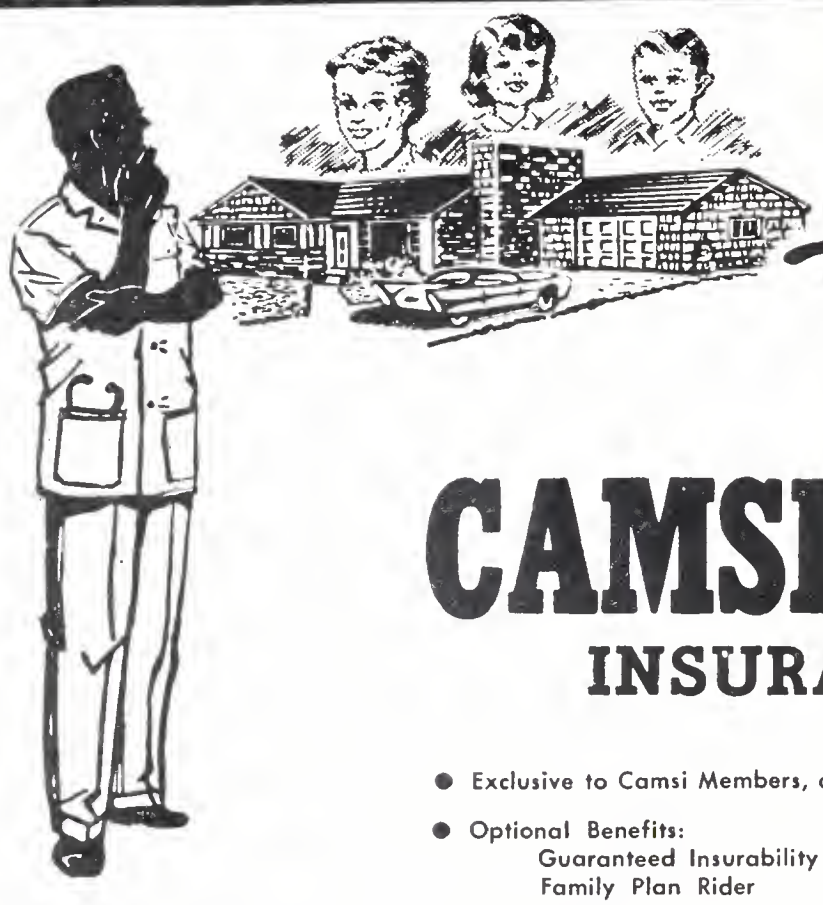

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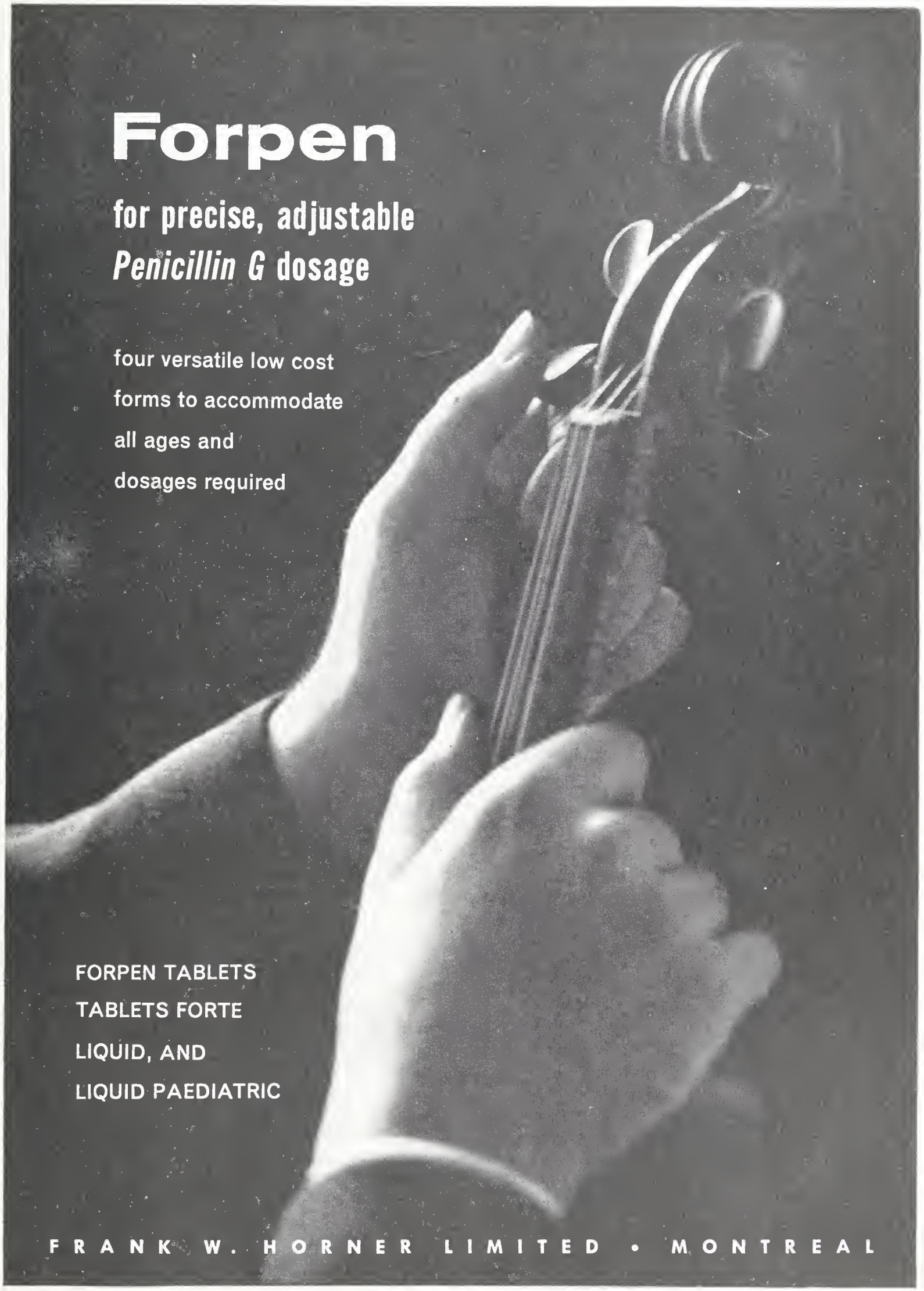
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INDEX

APRIL 1962

EDITORIAL 240

ARTICLES

WHY ARE THERE BLOOD GROUPS ..... *T. Edward Reed, Ph.D.* 243

CHRISTMAS DISEASE ..... *Nancy E. Simpson, Ph.D.* 249

PHENYLKETONURIA ..... *G. E. Connell, Ph.D.*[1]
B. P. L. Moore, M.B., B.Ch.[2]
M. W. Partington, M.B., Ph.D., M.R.C.P. (E) [3]
Norma Ford Walker, Ph.D., F.R.S.C.[4] 257

AUTORADIOGRAPHY AND IRRADIATION ..... *A. G. Bell, M.D., M.A.* 265

RISK TO MAN FROM RADIATION ..... *Diane Wilson Cox, B.Sc., M.A.* 273

THE THREE SYMPTOMS OF BREAST DISEASE ..... *R. P. Humphreys, 6T2* 281

FEATURES

BOOK REVIEW 255-271

THE COVER

In the beginning . . . The cell pictured on our cover is just completing a division (late anaphase of meiosis) whereby half of the potential of a parent is collected and stored as cellular material. This is the beginning of creation; two such collections will form another being. The man in the centre of the cell is another beginner. His name is Maupertuis. He could be called the father of human genetics, for, a century before Mendel, he investigated human heredity, applied mathematical laws to genetics, and finally formulated a theory of heredity involving analogous particles provided by each parent. From Maupertuis' beginning, the science of genetics has grown.

Thanks to Dr. Rothfels of the Botany Department, University of Toronto, for the photograph of the Trillium cell in anaphase.

INDEX TO ADVERTISERS

CANADIAN PREMIER LIFE ..... Inside Front Cover
FROSST ..... Inside Back Cover
SMITH KLINE & FRENCH ..... Outside Back Cover
HORNER ..... Page 235
ORTHO ..... Page 236
TAYLOR INST. ..... Page 238
OSBORNE'S PHARMACY ..... Page 241
PARK DAVIS ..... Page 242
POULENC ..... Page 248
WYETH ..... Page 256
J. SCHMID ..... Page 272
ONTARIO RETAIL PHARMACY ..... Page 279
CANADA LIFE ..... Page 280
LAKESIDE LABS. ..... Page 290

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Editorial

The existence of the University of Toronto Medical Journal is magnificent evidence supporting the fact that extra-curricular activity can play an important role in the scholastic life of a professional faculty. Those of us in the editorial and managerial staff of this publiction have been honoured by the interest in medical writing displayed this year by the undergraduates. For the first time in many years the contributions submitted were far more numerous than those we were able to accept. We hope that writing has been as rewarding an experience for our contributors as editing their manuscripts has been for us.

We were encouraged by the marked increase in subscriptions this year (225 new ones) which reflects widespread recognition for the efforts of the students in our staff during the past two years. Our readers hail from Russia, India, Nigeria, England and of course all parts of Canada and the United States.

As I step down from the editorship of this Journal I should like to express my gratitude to those students who have worked efficiently and tirelessly in the background in spite of being forgotten by the rest of the student body. My succesor, Jim Graham, whose good judgement and writing ability made him my right arm, was invaluable. The associate editors Christine Korsak, Robert Richards, Sidney Bender, John Brebner and the assistant editors David Cole, George McQuibban and Donald Strangway never reliquished their share of the editorial load. The managing editor, Bill Crysdale was the nerve center of the Journal's business operations. Without the efforts of Ken Ockenden in advertising and Fred Langer in circulation our whole project would have been a fiscal impossibility. The business manager Paul Korhonen and his assistant Peter McLeod reorganized the accounting system and spent long hours working on our accounts. Our art and photography expert, Mr. R. S. Gilder from Princess Margaret Hospital gave of his time freely and without his efforts the covers, *On Inspection* and *Diagnostic Radiology* would not have been possible.

There is room on our team. Share your abilities with us you will find that your efforts will have been profitably invested.

W.O.S.

This Issue

We would like to express our gratitude to Dr. Norma Ford Walker for organizing the graduate articles in this issue. It is hoped that these articles will serve to re-illustrate some of the principles of genetics, and their applications to medicine, as well as present some of the new work being done in genetics. The problems of geneticists in inherited diseases and conditions are suggested by the articles on Christmas disease and phenylketonuria. The role of genetics in medico-legal work is also hinted at in the latter article. Research in radiation effects plays a large part in the new frontiers of genetics, and this field is covered well by two articles. We hope the reader enjoys the 'graduate approach' for this last issue of volume XXXIX.

J.D.G.

ANNOUNCEMENT: From the Administrator's Office of the Wellesley Hospital.

The Wellesley Hospital, a division of the Toronto General Hospital for many years but now independent and one of the five teaching hospitals associated with the University of Toronto Faculty of Medicine, announces the launching of a ten million dollar building fund campaign.

The Wellesley Hospital has been continually expanding since its founding in 1912 as a private

hospital by Dr. Herbert Bruce, a well known medical practioner and one of the Lieutenant-Governors of the Province of Ontario. There were only one hundred beds in the first hospital, the total now is two hundred and eighty-five and when the building program will be completed in 1966 there will be six hundred and fifteen beds. In addition, research facilities will occupy 25,000 square feet in the new buildings. These facilities and the three hundred new beds reserved for teaching purposes will make the Wellesley Hospital a significant center of Canadian medicine. Some of the needed capital will be received from the government in the form of grants. The amount of $4,200,000 will be required from private sources to finish the project.

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Why Are There Blood Groups?

*by T. Edward Reed, Ph.D.**

This question is asked in all seriousness. Its answer is believed to be known and involves one of the less well known areas of human genetics. It is hoped that the background for this answer, although somewhat complex, as well as the answer itself, will have general medical interest.

In its evolution from the first decade of this century to the present, human genetics has progressed from demonstrations that some human traits are inherited, often in simple Mendelian ways, to more sophisticated activities. One of these is the study of population genetics, the behavior of genes in whole populations, in contrast to within a few individuals or families only. Population genetics describes the frequency of a gene, say gene *A*, and its alternate form (allele), say *a*, and the factors which cause these frequencies to change — mutation (e.g., production of new *A* genes from *a* genes), natural selection (loss of genes due to lowered viability or fertility), migration (of persons with new genes into the population), and genetic "drift" (chance deviation of gene frequency in children from that of the parents). If this field seems very remote from medicine, consider the new *mutations* induced by man-made ionizing radiation from X-rays or nuclear reactors or weapons, and the clinically-caused increase in frequency of the genes determining galactosemia and retinoblastoma, to name just two genetic diseases. These increases are due to the recently decreased *selection* against these diseases and hence against the genes (i.e., children who would previously have died from these diseases now may live; therefore, selection against the genes is reduced). It is worth emphasizing that genetically determined natural selection in man is *not* an abstract conception — the death of a child from cystic fibrosis of the pancreas or the infertility of an individual because of having Mongolism are two very concrete examples. This article will be concerned with one aspect, natural selection, of the population genetics of human blood groups. It will show that there is selection due to blood groups and will endeavor to answer the question of the title.

Associate Professor of Zoology and Paediatrics, Departments of Zoology and Paediatrics, University of Toronto.

The Problem

Of the twelve blood group systems known, only the ABO and Rh are of routine clinical importance, but as the labels on the blood bottles indicate, these are very important. The geneticist has always had a special fondness for blood groups because they are inherited in such a simple and clear-cut way, and also because the various groups within a system are usually relatively common, unlike most other simply inherited human traits, such as albinism or chondrodystrophy. Each blood group antigen on the surface of the red blood cell, such as A or B in the ABO system and D (Rh_o) of the Rh system, is determined by a dominant gene, i.e., it takes only one such gene to produce the antigen. Thus, if we call the gene producing the D antigen which makes a person Rh positive *R* and its allele *r*, and we know that each person has two Rh genes, then there are three types of persons: *RR*, *Rr*, and *rr*. The first two types (genotypes) are Rh positive, since at least one *R* gene is present. In our society the frequencies of these genotypes are about 36%, 48%, and 16%, respectively. The ABO system is more complex than this simplified interpretation of the Rh since there are three alleles instead of two: I^A, I^B, and I^O, *I* standing for isoagglutinin (antibody) and the superscript for the particular allele. A group A person is either I^AI^A or I^AI^O, a group B person is I^BI^B or I^BI^O, a group O person is I^OI^O, and a group AB person is I^AI^B. These relationships show that I^A and I^B are dominant to I^O. In our society the frequencies of O, A, B, and AB persons are about 45%, 40%, 10%, and 5%, res-

TABLE 1: Human blood group systems and Number of Commonly recognized blood groups in each.

System	No. of commonly recognized groups
ABO	4
Rh	*2 (only anti-D used)
MN	*3
P	2
Kell	*2
Kidd	2
Lewis	2
Lutheran	2
Duffy	2
Diego	2
Js	2
Xg	2

*Many more groups are known.

pectively. Other blood group systems, such as Kell or MN, have their own characteristic frequencies for their two or more types. With these preliminaries completed, we may now consider the question put by the title: why is everyone not group O, Rh negative, for example? Is there some real biological significance to the fact that there are four types of persons, with regard to the ABO system, two types (really more) in the Rh, two in the Kell, three in the MN, and so on? Or are these *polymorphisms* without any significance? I shall argue and try to prove that they are of considerable significance and have effects which can be clinically important.

When a gene has two forms, *A* and *a,* so that there are three genotypes, *AA*, *Aa,* and *aa,* and the *Aa* genotype, called the *heterozygote,* has a greater biological fitness (measured by number of children produced (than either of the other two genotypes (called *homozygotes*), there is a fundamental genetic principle that states that all three genotypes will be maintained in the population, generation after generation. As long as the fitness of each genotype remains constant, the heterozygote being most fit of the three, the frequencies of the genotypes will be constant. The existence of these three forms is called a polymorphism and their tendency here to remain at a constant frequency indefinitely makes it a *stable polymorphism*. In contrast, if the fitness were all the same, the polymorphism would be neutral and any chance effect on gene frequency, such as migration, could produce a change. And if a homozygote, say *AA,* were most fit, the allele *A* would become more and more frequent until it was 100% (barring mutation from *A* to *a*). There is one clear example of heterozygote superiority in man which leads to a stable polymorphism: individuals heterozygous for the gene which produces the abnormal hemoglobin called sickle-cell hemoglobin are more resistant to malaria. This appears to account for the high frequency of such persons in parts of Africa even though homozygotes for sickle-cell hemoglobin have a severe, often fatal, anemia. Such superior fitness of heterozygotes is well known in the genetics of experimental organisms, such as the fruit fly *Drosophila*. The point of all this is whether the converse applies: when there is a stable polymorphism, is the heterozygote necessarily more fit? If, as is done, "polymorphism" is defined to mean the existence of two or more forms (genotypes) in frequencies higher than can be accounted for by mutation (meaning, e.g., over 1%), then there is no known genetic mechanism, other than heterozygote superiority, which can produce a stable polymorphism. Therefore, the converse seems true.

The next question is: are the blood group systems stable polymorphisms? The answer, for some at least, seems to be "yes". All three alleles of the ABO system occur in all large populations and polymorphisms for two very similar alleles occur in certain anthropoid apes as well, suggesting a considerable antiquity of the ABO antigens. Both of the alleles of the MN system are present in all populations tested. The situation is less clear in other systems. But for the ABO and MN it is difficult to see why there should be polymorphisms all over the world unless there were some mechanism constantly operating to maintain them, i.e., keeping two or more alleles in the population. As indicated above, the only such mechanism known is superior hereozygote fitness and this is the answer, I believe, to the title — persons who are blood group heterozygotes, say *Rr* (Rh positive) individuals, are more fit, meaning that they are more likely to survive to adulthood and/or they have more children once reaching adulthood. All that remains is to prove this.

The discovery of the Rh blood groups in 1940 provided the first definite example of natural selection due to blood groups: Rh hemolytic disease of the newborn. The Rh+ red blood cells of the fetus, within its Rh— mother, are attacked by the antibody formed in the mother in response to fetal Rh+ cells passing through the placenta. There is no doubt that there is selection here — the only difficulty for our theory is that the fetus selected *against* is a heterozygote, receiving an *R* gene from its father and an *r* gene from its mother. This selection by itself would lead to the elimination of the *r* gene (since both an *r* gene and an R gene are lost in each such death and *r* genes are less common than *R*). If the Rh system is a stable polymorphism, which is not established, there must be other selection such that the heterozygote has an overall advantage.

A Search For Evidence For Natural Selection

To make selection more meaningful here, consider the following usual definition of biological fitness (natural selection value): The number of liveborn children ever to be born to a newborn liveborn individual. There are two components of this fitness: *viability* (survival from birth to the end of the reproductive period) and *fertility* (once adult). Both are required for this trans-

mission of genes from one generation to the next. Let us consider what evidence exists for each component. I shall briefly review work by other individuals and then discuss my own work at greater length.

Viability

In addition to the maternal-fetal incompatibility just mentioned (in both the Rh and the ABO systems), the other clear example of effect of blood group on survival is the association of various diseases with the ABO blood groups, as shown in Table 2. The most striking example

TABLE 2: Diseases showing strong associations with the ABO blood groups.

Disease	Relative Susceptibility
Duodenal ulcer	O/(A + B + AB) = 1.38
Gastric ulcer	O/(A + B + AB) = 1.19
Cancer of the stomach	A/O = A/B = 1.19
Pernicious anemia	A/O = 1.26
Diabetes mellitus	A/O = 1.16

is duodenal ulcer, group O persons being 38% more likely to develop this type of ulcer than persons of other ABO blood groups. This increased susceptibility of O persons has been found consistently in many different studies. There seems no doubt that there is a differential susceptibility to these diseases according to the blood group of the individual. What is not clear is the importance of these diseases to biological fitness (ability to produce children). All these diseases occur primarily in persons over 45 years, which is the effective end of the reproductive period, the proportion of deaths from these diseases which are under this age being only about 4% in American whites in 1955. These disease associations seem unlikely to explain the amount of selection needed.

Natural selection due to different susceptibility to certain *childhood* diseases, diptheria and whooping cough, for example, may have been important in the past. These diseases produced a high morality in the nineteenth and earlier centuries which was effective in natural selection. An association of blood group and childhood disease might produce appreciable selection. An example of such association may be provided by smallpox, which is not strictly a childhood disease: a serological similarity between variola virus and blood group A substance can be demonstrated and this leads to the prediction that A and AB persons, who lack anti-A isoagglutinin, might be more susceptible. One set of recent data from India appears to confirm this.

If there is some effect of ABO blood group on viability, a study of the frequency of blood groups at different ages might show this. Several studies have suggested such an effect but the largest such study, on 85,000 blood donors in England, did not show age differences. A study which I did in Michigan, on 15,346 individuals ranging in age from one to 85 years, was also negative for the ABO and also for the Rh blood group systems.

There is some evidence in the MN blood group system that there may be an excess of MN children from certain matings and, since these are heterozygotes, this would be an example of heterozygote advantage. This demonstration is difficult because there are many possible sources of error.

We may sum up these data by saying that although there are a few clear-cut demonstrations of an effect of blood group on survival, there are none, possibly excepting the MN, which show the heterozygote with an advantage. The ABO data, of course, give no information on heterozygotes (since an A person, e.g., may be I^AI^A or I^AI^O). As is so often the case, more data are needed.

Fertility

There have been only a few studies on possible effect of blood group on fertility and I shall summarize all of these, except the last, very briefly. This one is an extensive recent study which I carried out and which gave some new and surprising data.

In five different studies on the ABO system, three gave significant evidence for selection, of two studies in Rh, one gave evidence for selection, and the one study each in the MN and the P systems were negative. Of the three ABO differences in fertility, two clearly were due to ABO mother-fetus incompatibility (the mother has anti-A or anti-B and the fetus has the corresponding blood group antigen), the incompatible couples being less fertile, while the third study deviated in just the opposite direction. The significant Rh finding was obviously the result of hemolytic disease of the newborn due to mother-fetus incompatibility. None of these studies was ideal and in order to make a better assessment of possible selection due to blood groups a more elaborate study was planned several years ago and carried out in the town of Tecumseh, Michigan by Dr. Henry Gershowitz and myself. I was then in the Department of Human Genetics of the University of Michigan. The analysis of this study is still in progress.

The Tecumseh Blood Group Study

The critical study of fertility differences in modern North American populations is difficult because so many factors affect fertility. If we limit ourselves to essentially completed fertility by requiring the wife to be, say, 40 years or older, the more important factors include desire for children, use and effectiveness of contraception, duration of marriage, education of husband and wife, occupational status, economic status, religious affiliation, and birthplace of husband and wife. These factors are not independent of each other and are of greatly differing importance. Statistical techniques, carried out by electronic computer, however, enabled one to "remove" the effects of a number of these influences before examining the data to see whether persons of different blood groups differ in their fertility.

Tecumseh is a town of about 8,000 in southeastern Michigan and was chosen for this study because it had been previously studied in a long-term, still-continuing investigation of morbidity by the School of Public Health of the University of Michigan. A list of all couples participating in this study in which the wife was 40 or more years of age, about 850, was the starting point for the blood group investigation. A trained field worker tried to contact each couple in order to obtain personal and reproductive histories and also take finger-prick blood specimens and saliva specimens. The type of information obtained on each of 754 couples is listed in Table 3. This information was coded and put onto IBM punch cards for analysis.

TABLE 3: Data obtained on couples in Tecumseh blood group study

Personal Data (H = husband, W = wife)
Birthdate — H, W
Birthplace — H, W
Education, years — H, W
Number of sibs — H, W
Religion — H, W
Income — H
*Occupation — H
Age at marriage — H, W

*10 categories, ranging from professional to laborers.

Reproductive Data
Date and outcome of each pregnancy of this marriage (No. of pregnancies, livebirths, spontaneous abortions, induced abortions, stillbirths) (Abortion = fetal death under 20 weeks gestation)

Blood Group Data
ABO (O, A_1, A_2, B, A_1B, A_2B)
Rh (using anti-C, c. C^w, D, E, e)
MN (M, MN, N)
P (P+, P−)
Kell (Kell +, Kell −)
Kidd (Jka+, Jka−)
Duffy (Fya+, Fya−)
ABH secretion in saliva (secretor, nonsecretor)

The preliminary analysis employed the statistical technique known as multiple regression analysis to determine, and remove, the effects of the personal data variables of Table 3 on each of the following four reproductive variables: 1) number of pregnancies, 2) number of livebirths, 3) proportion of pregnancies terminating in spontaneous abortions, and 4) proportion of pregnancies, not ending in spontaneous abortions, which terminate in stillbirth. This procedure has the effect of standardizing each couple to the same value for each personal data variable, such as wife married at age 20, husband with 12 years of public school, both partners Protestant, etc. It seems the only way to correct for the unavoidable heterogeneity of human populations. This procedure, incidentally, yielding interesting data on the selective magnitudes of the effects of these social variables, including unexpected findings such as a significant effect of husband's education on numbers of pregnancies and livebirths but no significant effect due to wife's education!

Having standardized the couples, the possible effect of blood group on each of the four reproductive variables was tested by the method of analysis of variance. This is the most general approach and, in the way used here, consisted of determining how much of the variation, from couples of one blood group combination to couples of another combination, in the value of the reproductive variable was due to differences in husband's blood group alone, wife's blood group alone, and blood group of husband and wife together. Comparing each of these three amounts of variation between blood groups with the variation occurring *within* classes of couples of the same blood group allows the significance of each to be determined. Thus, in analyzing pregnancies, it was found that this ratio of "between blood group" variation to "within blood group" variation was 3.4 for husbands, 1.6 for wives, and 0.7 for couples: the husbands' ratio is "moderately" significant, the others are not, and it is therefore possible that blood group of the husband affects fertility (wives of group O men having more pregnancies). This finding must be confirmed before being accepted. The results of these 32 analyses (8 blood groups x 4 variables), each with three components, yielded five findings significant at the 0.01 level. (A result is called "significant" here if the probability of its occurring by chance, i.e., not due to an effect of

blood group, is less than one in a hundred.)

These are:

1) Wife's Kell group – no. of pregnancies
2) Wife's Kell group – no. of livebirths
3) Wife's Rh group – proportion of pregnancies terminating in spontaneous abortions
4) Couple's Rh group – proportion of pregnancies terminating in spontaneous abortions
5) Wife's Duffy group – proportion of pregnancies terminating in stillbirths.

Since numbers of pregnancies and livebirths are highly correlated, results 1) and 2) are essentially the same, in each case Kell negative women being more fertile than Kell positive. Standardized to the average couple, the mean number of pregnancies for the former is 2.95 and 2.04 for the latter. This large difference is quite unexpected: there are no other data on this point and confirmation is required. The two Rh findings appear to result from an increased proportion of abortions in Rh negative women (0.128), very likely due to Rh hemolytic disease of the newborn. This result, in a *normal* population, appears to be a nice confirmation of the well-known clinical sequelae of maternal-fetal Rh incompatibility. The relation between wife's Duffy group and proportion of stillbirths is also unexpected and requires confirmation. Duffy (a+) women appear to have a higher proportion of stillbirths, 2.1% instead of 0.8% for Duffy (a–) women.

The demonstration of these effects of blood groups is quite distinct from their genetic significance. Unfortunately, for the sake of our theory, none of these five effects supports the hypothesis of superior fitness of heterozygotes. The more fertile Kell negative women are homozygotes and the Rh and Duffy results give no definite information on the heterozygote. We are left in the interesting but unsatisfactory position of having some new data on selection due to blood groups which do not explain the polymorphism of blood groups.

Conclusions

There seems no doubt that natural selection can be due to individuals of one blood group being more biologically fit than those of another. The Tecumseh study, as well as some earlier ones, make this quite clear. But there are no clear-cut data showing heterozygotes more fit than either homozygote and this relationship is the only situation known to produce stable polymorphism. At the same time, it must be remembered that each study has considered only one aspect of selection, either viability or adult fertility, and it is the overall fitness over the whole life cycle which counts. It may be that the present blood group polymorphisms are the result of the pattern of natural selection which existed 50 or more years ago but which no longer exist. Or perhaps the selective differences, over the life cycle, are only a few percent, too small to be detected by studies of the type made so far.

To go back to the title, there are blood groups because they are, or recently were, a stable polymorphism maintained by heterozygote selective advantage. The somewhat contradictory data do not disprove this; unfortunately, they do not prove it either. There will still be some work to be done here after the first man reaches the moon.

UPJOHN ABSTRACT: *Anorexia for Alcohol: A Side-Effect of Phenethylbiguanide* (Letter to the Editor) Lisboa, P. E. Castel-Branco, N. Sá Marques, M. Lancet I:678 (March 25) 1961.

A curious side-effect of phenformin was first reported to the writers by a chronic alcoholic with diabetes. During the first few days of phenformin treatment, even with the initial dose of 75 mg. daily, he had such a dislike for alcoholic drinks that he stopped them altogether. Other side-effects such as anorexia and postprandial satiety did not develop until the third week. He remained totally abstinent for more than 3 months without other anti-alcoholic treatment, but he resumed his consumption of alcohol after phenformin was withdrawn.

The writers have inquired about this side-effect of 50 diabetics who have been given phenformin. Of the 33 diabetics who had been drinking alcoholic beverages more or less regularly, 10 showed this side-effect in various degrees. The unpleasantness on drinking alcohol generally appeared during the first days of phenformin treatment and on relatively low doses (75 to 150 mg. daily). In some instances the loss of desire, or even repugnance, for alcoholic drinks was associated with their metallic taste.

A trial has been started among alcohol addicts. Such an effect of phenformin might be used therapeutically for alcoholism in general, since it seems that healthy people are resistant to the hypoglycemic effect of phenformin.

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Christmas Disease

*Nancy E. Simpson, Ph.D.**

Interrelationships between genetics, biochemistry and medicine are becoming increasingly important and indeed, essential for the complete understanding of disease. "The growing awareness that the primary activity of a gene is the control of specificity of a substance such as an enzyme is adding immeasurably to the precision of the various practical applications of medical genetics."[1] Snyder might have stated that the concept of gene activity contributes to the precision of applications of *genetics* to *medicine*. Further subdivisions of a clinical classification have been made from increased knowledge of the genetics and biochemistry of a disease, just as more careful clinical classifications of a disease have stimulated investigators to look further into the genetics and biochemistry of the disease. The purpose of this paper is to show how the science of genetics has contributed to the knowledge of disorders of blood coagulation in general and to the knowledge of Christmas Disease (P.T.C. deficiency, or Factor IX deficiency) in particular. It is not possible to develop the story of the contribution of genetics to this field without including the role played by biochemistry.

Bleeding Disorders

Bleeding disorders may be attributed to vascular or blood platelet abnormalities or to defects in the clotting mechanism[2]. Although the present classification of diseases of the latter group is not based on precise chemical knowledge of the particular clotting factor (excepting Ca^{++}), the reactions which they undergo or catalyse are known phenomenologically[3]. Many of these diseases which are a result of a clotting factor deficiency have a genetic basis. Thus, genetic information plus the knowledge that a certain fraction of normal plasma or serum will correct clotting in the deficient plasma or serum, have been used as a basis for classification of disorders of clotting deficiencies.

Haemophilia

In the present paper, only the best known and most common of the clotting defects, haemophilia, will be discussed. Biggs and Macfarlane[4] point out that for many years haemophilia was defined mainly by clinical features and inheritance.

Writings in the Talmud by Rabbi Simeon ben Gamaliel[5] who lived in the second century A.D., imply that he recognized the familial tendency of bleeding after circumcision. The Rabbi exempted a woman from circumcising her son because three of her sisters had had sons who died after the operation. Nasse[6] noted that bleeding disease was transmitted by unaffected daughters of bleeders to their sons and much of the subsequent literature regarding this disease confirmed this type of transmission in families. In 1828, Hopft[4] introduced the name haemophilia for bleeding disease. After Morgan and Bridges[7] showed that this pattern of inheritance in *drosophila* was caused by genes located on the X chromosome, it was realized that haemophilia was transmitted by an X-linked recessive gene.

For many years haemophilia was the only "bleeding disease" known to be X-linked recessive. The deficient factor known as antihaemophilic globulin (AHG or AHF) was incorporated into the theory of normal physiology of clotting as a substance necessary for the formation of blood thromboplastin. There is still little information regarding its chemical properties. In 1952 independent investgators in England[8] and in the United States[9] found patients whose serum when added to the serum of another of their haemophilic patients, improved the latter's clotting time just as readily as did the serum of a normal individual. These new patients had family histories indicative of X-linked recessive inheritance, so it was concluded that although the missing blood coagulating factor must be different in the two types of haemophilia, the genetical transmission was the same. The new type of haemophilia was given the name of Christmas Disease by Biggs et al[8] and plasma thromboplastin component deficiency (PTC) by Aggeler et al[9]. Subsequent knowledge of the properties of Christmas factor and PTC have shown that the factor lacking in these two patients was the same, and genetic studies have confirmed that this deficiency is controlled by an X-linked recessive gene. The observation that the affected relatives of a patient with Christmas disease also have Christmas factor deficiency and not AHG de-

**Department of Pharmacology, University of Toronto.*

ficiency, led to the hypothesis that the gene for Christmas disease must be different from that which controls classical haemophilia. It should be mentioned that an occasional exception to this rule has been observed, in which both types of haemophilia have been found in one individual or one family, but these are rare and can be attributed to chance rather than suggesting that one gene is controlling the two different deficiencies in one family[10-17].

There is evidence that AHG deficiency may be further subdivided by the fact that mild and severe cases do not seem to occur in one family, and possibly there are different genes for different relative amounts of the clotting factor[18]. In a more recent publication, Graham[19] points out that relatively low AHG levels in mild haemophilic patients are often associated with a vascular defect causing long bleeding time and this condition may be a different entity inherited as an autosomal dominant. At this time, it is not clear whether all mild haemophiliacs with AGH deficiency belong in this category.

Christmas Disease

Recent extension of knowledge of the genetics of Christmas disease[20] in a study of a number of families, will be used to illustrate some of the general concepts discussed above.

Since it was well known that Christmas disease occurred clinically in mild and severe forms, genetics was used as a tool along with a quantitative assay of Christmas factor activity to determine whether Christmas disease could be classified as more than one entity. In addition, genetics was used as a tool to distinguish women who were heterozygous for the Christmas disease gene in families of Christmas disease patients. Using the assay of Christmas factor, the amount of Christmas factor in the heterozygous or genetic carrier women could be compared with that of normal women for the purpose of distinction between these two genetic types. It is obvious that the detection of such heterozygous women would be valuable to the clinician in predicting the chances for their sons to be affected. In other words, genetics is contributing to the development of a test for the detection of sub-clinical genetic carriers of the condition.

The study consists of results obtained from data gathered from 16 families living in and around Oxford, England. All of the families in which there was a patient with Christmas disease were probably ascertained and studied. Most cases in the area were referred to Dr. Biggs and any who may not have been, were ascertained from a request to all general practitioners (whose response was 95%) and Medical Officers of Health in the area and through the Haemophilic Register. Since the prevalence of Christmas disease is rare and considered to be about 1 to 3 per million, this is quite a large number of families.

It was first noted that males who were severely affected with Christmas disease by clinical standards, had no detectable amounts of Christmas factor activity in their sera, as tested by a modified thromboplastin generation test[21], whereas the males who were mildly affected had Christmas factor activity from 1 to 11% of normal pooled plasma. The clinically severely affected males, whose sera had no detectable amounts of Christmas factor, are less likely to survive for very long and only six families were found of this type. In one of the six families, there was one living affected male in addition to the six probands and his serum also had no detectable Christmas factor activity. Other affected members who were not living, in the six families, however, were clinically severe cases and died at a relatively early age. On the other hand, in the ten families for which the probands showed mild clinical symptoms and their sera had *some* Christmas factor activity, all other affected males were mildly affected and their sera had some Christmas factor activity. The number of living affected males in these families ranged from 1 to 10 and although considerable variation in amount of Christmas factor activity occurred in sera from any one family, some of the variation was shown to be due to age of the individual[20]. The important fact remains that all of the affected males in these families had detectable amounts of Christmas factor and were clinically mild cases. A large family in which there were 10 living affected males is illustrated in Fig. 1 and their Christmas factor levels and age are tabulated by generation and pedigree numbers in Table I.

This is an extremely rare condition and the observation that all the affected members of some families are clinically severe cases in whose sera no Christmas factor activity is detectable, whereas in other families all the affected members are clinically mild cases in whose sera slight Christmas factor activity is detectable, suggests that genetically there are two types of families. Two different genes or alleles may be implicated although both genes must be X-linked or other genes modifying the amount of Christmas factor produced by the

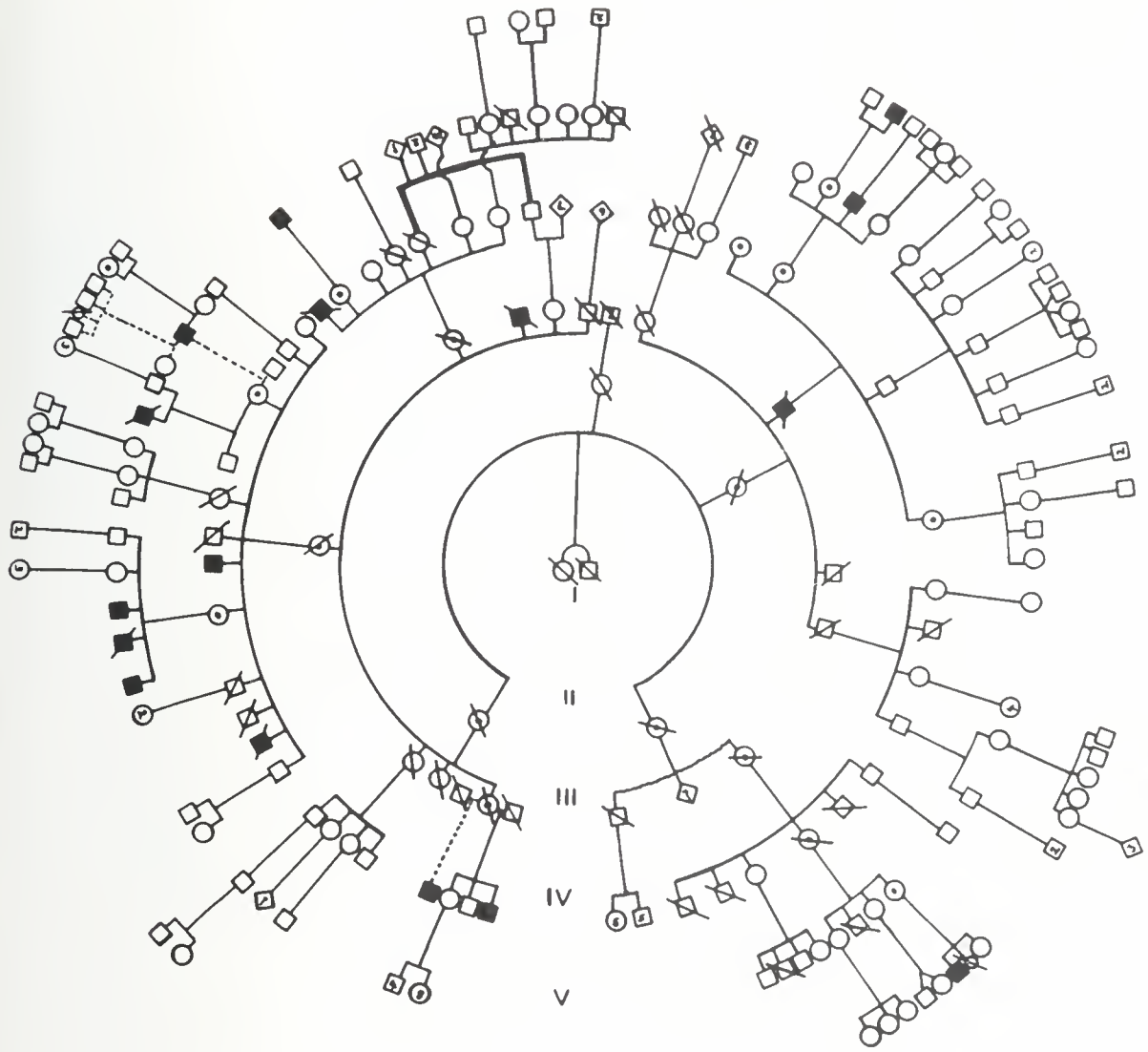

Fig. 1 — Christmas Disease Pedigree

Legend

- normal male
- normal female
- affected male
- heterozygous female
- sex unknown
- miscarriage
- deceased
- consanguinous marriage
- order in sibship unknown
- unmarried

Individuals in text are referred to by Arabic numbers reading anticlockwise beginning at 1 to right of generation number designated by Roman numerals.

From Simpson and Biggs (20); reproduced by the courtesy of the publishers.

TABLE I: Christmas Factor Levels and Age for affected males and their proven heterozygous female relatives in the family illustrated in Figure 1.

Affected Males			Proven Heterozygous Females		
Pedigree Number	Age	C.F.* Level	Pedigree Number	Age	C.F. Level
IV 66	73	7	IV 21	72	27
IV 56	59	7	IV 52	67	53
IV 69	59	8	IV 23	65	32
V 59	42	5	IV 47	63	57
V 28	38	7	IV 24	63	27
V 56	35	4	IV 57	61	93
V 68	22	2	V 29	31	73
V 70	17	2	V 8	31	56
VI 35	11	2			
VI 6	5	8			

*C.F. — Christmas factor level (per cent of normal pooled plasma).

After Simpson and Biggs (20); reproduced by the courtesy of the publishers.

major gene, may be present. From a chemical point of view different changes in the molecule of Christmas factor may be the cause of lack of activity or relative loss of activity. It is evident that clinical and Christmas factor level differences, which are fairly consistent within families, should stimulate biochemcal research into the nature of Christmas factor and when precise chemical knowledge is available, the geneticist will be able to clarify details in his field.

Genetic Carriers of Christmas Disease

The two types of haemophilia have been said to be inherited by an X-linked recessive gene, but the gene is not always completely recessive in heterozygous females. Instances have been reported for both AHG deficiency[22] and Christmas factor deficiency[20] in which some of the females have exhibited mild clinical symptoms although for both conditions these symptoms are not constant in all females who are heterozygous for the gene in question. In this paper, the problem of lack of recessiveness will be discussed for Christmas factor only.

There are five possible genotypes when an X-linked gene is considered. Males who have only one X chromosome (which pairs with their Y chromosome) have a gene for normal or a gene for Christmas disease and are classed as normal or affected. They have already been discussed. Females have two X chromosomes. Those females with two genes for normal are classed as normal; those with two genes for Christmas disease as homozygous for Christmas disease, and those with a gene for normal and one for Christmas disease as heterozygotes or genetic carriers. Only two of the three possible genotypes were available for testing. The female homozygote for the Christmas disease gene by theory, would occur $(1/1{,}000{,}000)^2$ or 10^{-12} and was not observed in the present sample. In fact, a female who is homozygous for the Christmas disease gene would not occur in every generation of the world population (250 to 300 million). On the other hand, the rarity of the gene allows us to assume that women with no symptoms and no family history of bleeding diathesis are homozygous for the normal gene and these could be compared with women in the Christmas disease families who are proven to be heterozygous on genetic grounds. Such a comparison would indicate whether female heterozygotes can be distinguished from normal homozygotes. In other words, the degree of recessiveness of the gene can be assessed.

A female control of 48 normal women used in the study consisted of 26 staff members of an Oxford hospital, 7 females in the families who were either unrelated wives of affected males or daughters of unaffected males, and 15 patients attending medical clinic at the Radcliffe Infirmary, Oxford, who had no known blood dyscrasia themselves or in their families. The criteria for proven heterozygous females in the families were that the women must be: (a) daughters of affected males; (b) mothers of more than one affected son; (c) mothers of more than one daughter with affected progeny; (d) mothers of an affected son and a daughter with affected progeny; (e) mothers of one affected son or one daughter with affected progeny who had some other affected relative. Criteria (b), (c) and (d) are based on the assumption that a mutation which occurs in primordial germ cell material and which could give rise to a mother transmitting the gene to more than one of her offspring, is considered rare enough to be disregarded. For the family illustrated in Fig. 1, these heterozygotes are indicated and the reader can see for himself how a considerable number of female heterozygotes can be proven on genetical grounds from such an extensive pedigree. For example, the mother (V 8) of the affected male (VI 6) could not have been established as a genetical carrier until considerable study of church and civil records revealed that her great grandmother (II, 1) was a sister of II, 2 and II, 4, who were women who had transmitted the Christmas disease gene to their progeny.

Results of the assay of Christmas factor activity indicated that in all four genetic groups there was a tendency for Christmas factor activity to rise slightly with age. Age and Christmas factor levels are given in Table I for the female heterozygotes from the family illustrated in Fig. 1. Although these data do not indicate a correlation between Christmas factor level and age, there was a correlation when data for all the heterozygotes were pooled. Further details of these results are published elsewhere[20]. Even though the mean level of Christmas factor activity in 53 female heterozygotes was significantly lower (55.3%) than that in the 48 female controls (97.3%) the distributions of the two groups overlapped (Fig. 2) to such an extent that only 13% of heterozygotes could be distinguished with certainty by this method. The extreme variability of Christmas factor level in heterozygous women in a family may be seen in Table I.

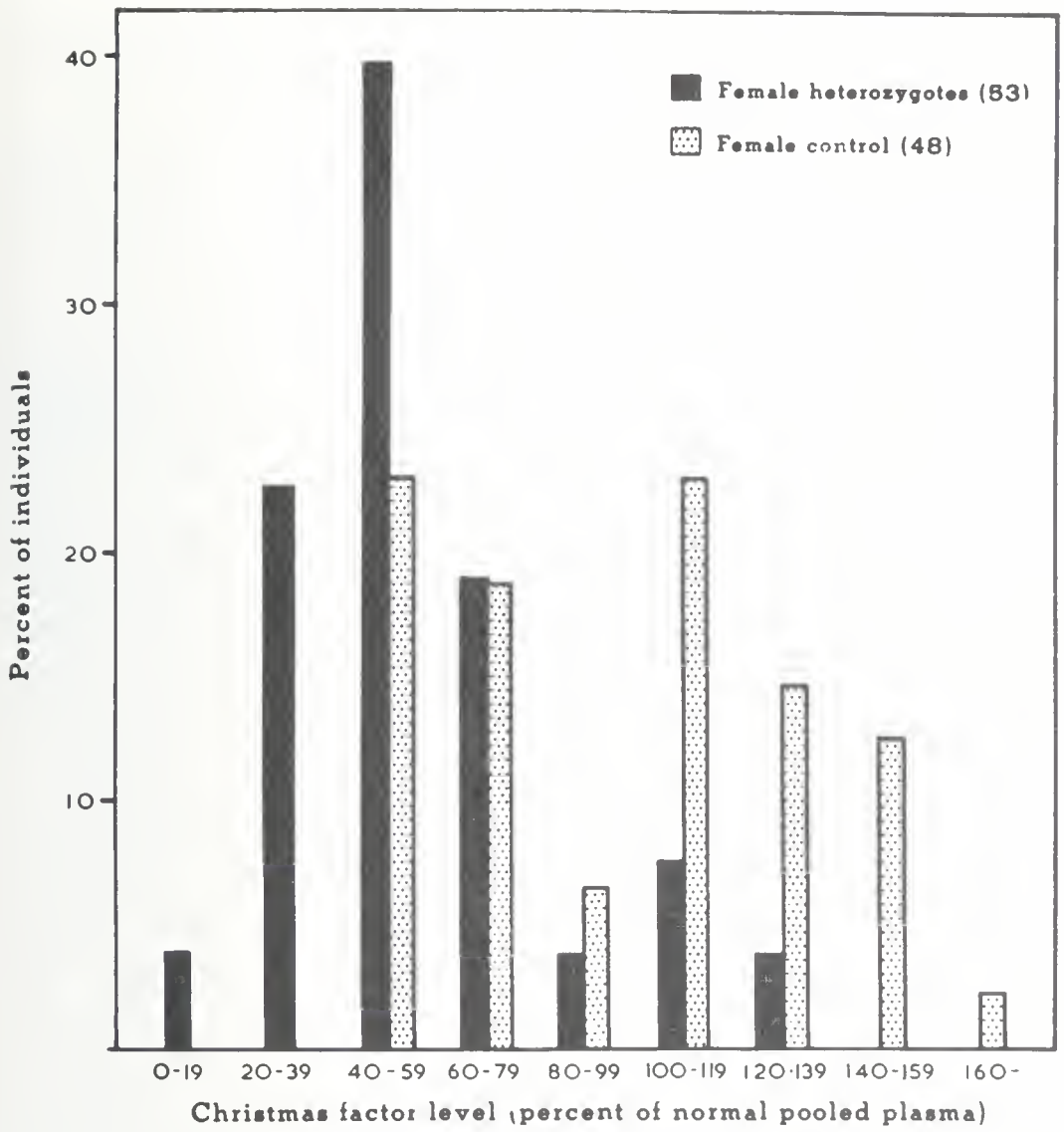

Fig. 2 — Distribution of Christmas factor levels in female heterozygotes and controls.

Redrawn from Simpson and Biggs (20) by courtesy of the publishers.

The results obtained by testing female heterozygotes do not necessarily indicate that the method of assay is not valid. The degree of accuracy for the majority of observations has already been shown to be ±15%[21]. Since the assay measures activity and perhaps not the actual amount of Christmas factor, this may be one reason for failure to demonstrate clear cut differences between heterozygous and normal women. On the other hand, the results show considerable variability in activity of Christmas factor within the genetically homogenous groups. It is of some interest to note that the distributions of Christmas factor activity produced by a single gene in 71 normal males (range is 32-170%) and by two genes in 48 normal homozygous females (range is 40-166%) are similar with mean levels of 96.3% (standard deviation ±32.9%) and 97.3 per cent (standard deviation ±35.4%) respectively. The levels produced by one gene for normal and one for Christmas disease in 53 heterozygous women had a range of 11 to 130%, whereas those levels for one gene for Christmas disease in 43 affected males had a range of 0 to 11%. The combination of one normal and one Christmas disease gene in one heterozygous female produced the same low activity (11%) as that of one Christmas disease gene in an affected male (from another family). The heterozygote was the daughter of a Christmas disease patient and her mother was presumably normal with no history of bleeding in her family. Unfortunately it was not possible to test her mother. The daughter was probably not homozygous for the Christmas disease gene. If she were homozygous, her mother would have to be heterozygous for the Christmas disease gene which is unlikely or a mutation in the gene from her mother would have to occur, an even less likely event. In another family, a female's serum had Christmas factor activity of an exceptionally low value. This child could not be classified as a proven heterozygote on genetical grounds as she was a daughter of a known heterozygous woman but had half a chance of carrying the gene. The very low level of 3 per cent in this 11 year old girl (confirmed on two different occasions) suggested that she might be homozygous for the Christmas disease gene. Her father had a level of 122 per cent and if she were homozygous, the Christmas disease gene from her father must be the result of a mutation. Again, the probability for a mutation is extremely low. She had 46 apparently normal chromosomes, which excludes the

possibility that she was an XO individual, although a small deletion on the X chromosome involving the Christmas disease gene would not be detectable by present cytological methods.

Understanding of different dose effects of this gene will have to await further chemical knowledge of Christmas factor which could give more conclusive evidence as to whether there is one or more than one gene for Christmas disease. Dose effects of genes are known to produce rather unexpected results in lower animals[23]. If more than one gene for Christmas disease is implicated, the variation in levels may be a reflection of different gene actions and not differences in dose effects.

Summary

A genetical study of Christmas disease along with the measurement of the activity of the deficient factor and clinical information has been used as a tool to learn more about the disease. The data suggested that the entity may be subdivided and have shown that by using activity as a measure of the deficient factor, some conclusions, although tenuous, can be made regarding gene action. It is obvious that much more satisfactory conclusions for both of these points must wait precise chemical information of Christmas factor.

ACKNOWLEDGEMENT: The data, Figures and Tables are reproduced with the kind permission of Dr. R. Biggs, and Blackwells, publishers of the British Journal of Haematology. The support of the Queen Elizabeth II fund for this work is also acknowledged.

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UPJOHN ABSTRACT: *The Rationale for the Treatment of Ischemic Heart Disease with Anticoagulants.* McDonald, L. Dis. Chest 39:317-322 (March) 1961.

Blood coagulation in ischemic heart disease was the subject of extensive investigations by the author, published from 1957 to 1960 and reviewed here. They clearly demonstrated, for the first time, hypercoagulability of the blood in patients with ischemic heart disease. They confirmed the hypercoagulability of the blood described previously in patients with angina pectoris. Hypercoagulability was greater after cardiac infarction. The findings agree fully with the clinical observation that patients with ischemic heart disease have an increased tendency to thrombosis. The data upon which these conclusions were drawn were the results of 10 different tests of blood coagulation performed on patients and controls under strictly uniform conditions.

The various anticoagulants do not act alike in restoring blood hypercoagulability towards normal. Heparin rapidly depresses thromboplastin-generation to normal levels and below and has a lesser effect in reducing platelet-stickiness towards normal. Phenindione affects both these tests to a lesser extent than heparin. Since prothrombin time is usually used to govern phenindione dosage, but the amount of anticoagulant needed probably varies with the degree of hypercoagulability, it is not surprising that thrombosis develops in some patients in whom the prothrombin time is well controlled with phenindione.

Book Reviews

Atlas of Hematology, 91 Pages, 256 Colour Illustrations, Sandoz Ltd., 220 Metropolitan Blvd., Dorval, P.Q., $5.00.

That a picture is worth a thousand words, is amply verified by this Atlas — a book designed to facilitate the learning of various hematological specimens with it, the student and practising physician alike can identify without difficulty the corpuscles of the blood and hematopoietic organs. The diagrams and pictures are excellent, the blood cells being faithfully portrayed, their appearance being that actually seen when a *well* prepared film is examined under the microscope.

The Atlas is divided into three sections, the first is termed General Considerations and includes, fundamental principals of hematology, technique of blood and bone marrow examination etc. The second section is concerned with the morphology of the blood corpuscles and the third section is comprised of colour plates. Since hematology is no longer the hobby of specialists but an indispensable diagnostic aid to the modern physician, this text is well worth the investment.

F.L.

Textbook of Endocrinology, by Robert H. Williams, 1204 pg., W. B. Saunders Co., 1962, $22.05.

Since thousands of papers on endocrinology and metabolism are published annually, the incorporation into one book of the most pertinent information presented in a clear, concise, and authoritative manner is most important. This text is an encyclopedia of endocrinology each hormone being discussed from every aspect. For example the section concerned with the neurohypophyses includes these topics: anatomy, characterization of hormones, biologic actions of neurohypophyseal hormones, neurohypophyseal hormones in blood, diabetes insipidus, vasopressin excess, and references. The ovaries and pancreas and other endocrines are also brilliantly discussed. Sections are included on general principles endocrine problems in children, the relation of hormones to cancer, the relation of genetics to endocrinology, laboratory tests and techniques of diagnosis and treatment.

The various hormones are dealt with by various eminent authors, most of whom have engaged in both basic and clinical investigations and are adept in the practical application of basic knowledge to clinical problems. For the student, practising physician, or researcher, who desires a stimulating, comprehensive and rewarding text of endocrinology, this book is the answer.

F.L.

Management of Hypertensive Diseases, by Joseph C. Edwards, A.B., M.D., F.A.C.P., F.A.C.C., Publication date; November 1960, by C. V. Mosby Co., 439 Pages, $15.00.

"At present, our methods of treatment of hypertension are not specific because so little is known about the fundamental mechanism of the disease". The preceeding is a quote from Dr. Edwards' book *Management of Hypertensive Diseases.* In this book the author has attempted to further our knowledge by correlating all the known facts about the common and more rare types of hypertension.

The first part of this book deals with a general discussion of the condition, methods of diagnosis, techniques of blood pressure measurement, and a general regimen of treatment including dietary management. Dr. Edwards discusses in some detail the conditions associated with hypertension, special diagnostic tests and aids plus the mechanism of action, usefulness and toxicity of the presently available hypertensive drugs.

The author has reviewed the majority of papers written on the subject, 60 pages of his book listing the references he has used. The organization is unsatisfactory: selected subjects seem to be scattered throughout the book, but the index is adequate.

This book is not recommended for the undergraduate student, but it may be of use to the clinician, and it would be a valuable asset to research worker interested in hypertension since Dr. Edwards has so completely reviewed the literature on the subject.

W.W.A.

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Phenylketonuria in One of Twins

by *G. E. Connell, Ph.D.*(1)
B. P. L. Moore, M.B., B.Ch.(2)
M. W. Partington, M.B., Ph.D., M.R.C.P. (E) (3)
Norma Ford Walker, Ph. D., F.R.S.C.(4)

Beware of pitfalls in the use of the Twin Method in medical research. The case reported provides an opportunity to review the criteria by which the type of twinning is diagnosed. The case has also a number of interesting features in the clinical picture of phenylketonuria.

Phenylketonuria is an inborn error in the metabolism of phenylalanine in which the main clinical features are severe mental retardation and the excretion of phenylpyruvic acid in the urine. The basic lesion is a deficiency of the enzyme phenylalanine hydroxylase. In consequence, phenylalanine accumulates in the blood and cerebrospinal fluid and phenylalanine and its metabolites are excreted in excess in the urine. The altered phenylalanine metabolism is now thought to be responsible for the retardation because if the abnormal metabolism is controlled from early life by a low phenylalanine diet, the child will develop normal or near normal intelligence.

Phenylketonuria is believed to be inherited through a single, completely recessive, rare, autosomal gene with full penetrance. The present family conforms to the criteria of such a mode of inheritance, in that: (1) both parents are normal and heterozygous and the more remote ancestors are also normal; (2) within this family unit the metabolic error has repeated among the children; (3) both sexes are affected; (4) the increase in the incidence of consanguinity among the parents of affected individuals is here observed in a third-cousin marriage.

(1) *Assistant Professor of Biochemistry, University of Toronto.*
(2) *Director of National Laboratories, Canadian Red Cross Blood Transfusion Service, Toronto.*
(3) *Department of Paediatrics, Queens University, Kingston. (Formerly Research Fellow, Hospital for Sick Children, Toronto).*
(4) *Professor of Human Genetics, University of Toronto and Director, Department of Genetics, Hospital for Sick Children, Toronto.*

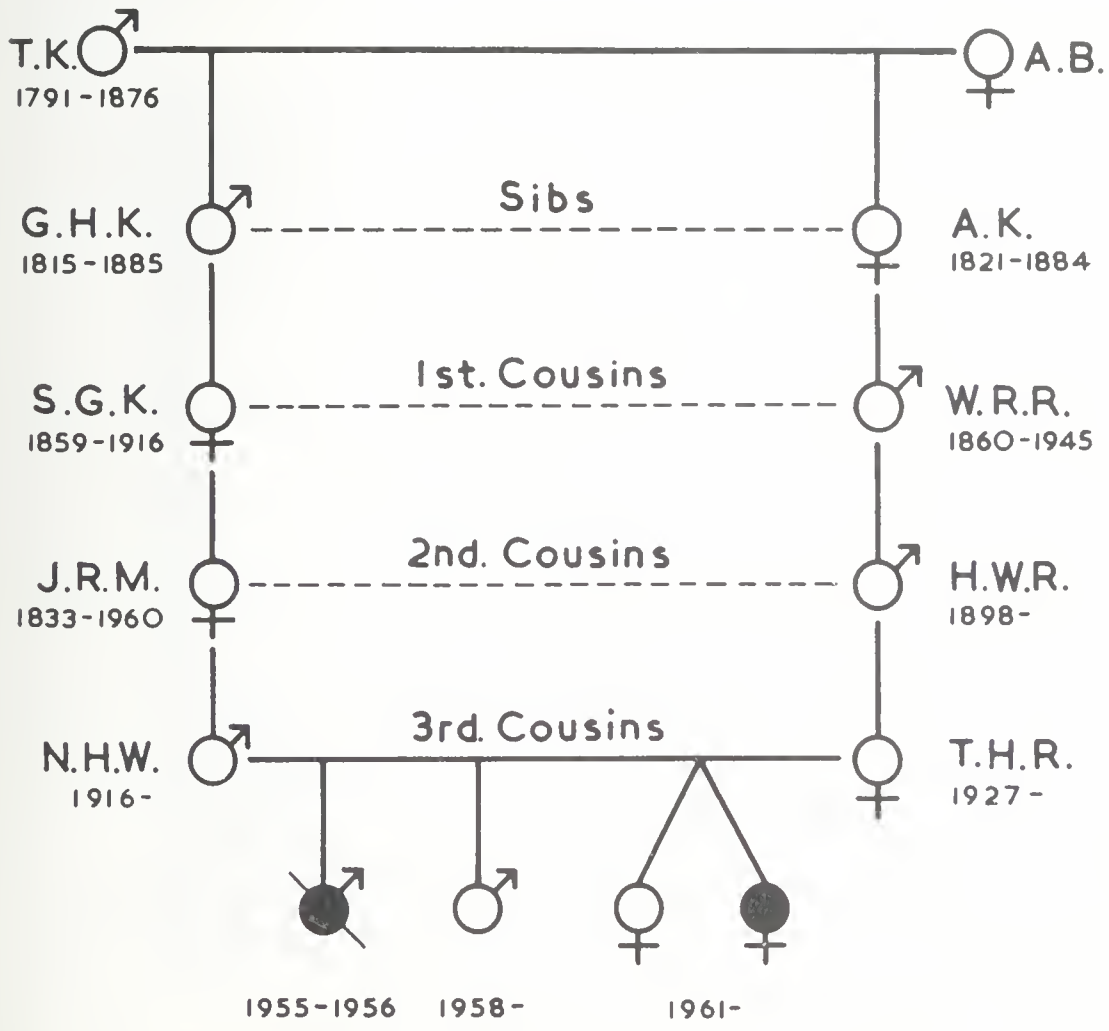

Fig. 1

Pedigree of the W-family showing the consanguinity of the parents.

The Twin Method is valuable as a test for the full penetrance of the gene for phenylketonuria. If the twins were a monozygotic set (which in the first weeks of life they were thought to be) then both members should be affected. Since only one twin was affected considerable speculation resulted concerning complete or incomplete penetrance of the gene, or whether there was error in the determination of the zygosity of the twins.

Family History

The Canadian family here described belongs to a distinguished pioneer family of United Empire Loyalists who have documented and published their pedigree over nine generations from 1759 to the present. The records are complete with dates and marriages for more than 644 members. Such records are invaluable for genetic research. Indeed it was not until the extensive pedigree records were assembled for publication that the parents of the family here discussed, learned that they were third cousins. Presumably the rare recessive gene for phenylketonuria in this family has come from only one source and has been transmitted to the third cousins from a common ancestor, that is from either their great-great-grandfather (T.K.) or great-great-grandmother (A.B.) to express itself in the homozygous affected children of the sixth generation (Fig. 1).

The first child, William, was born in 1955 and did well until the age of two months when a red, scaly rash appeared on his face and spread to the rest of his body. Shortly after this he began to vomit and this was sometimes projectile in character. At the age of four months, he was admitted to the Hospital for Sick Children, Toronto. He was found to have infantile eczema, a small head (circumference 38 cm.), mental retardation and a mouse-like smell. His urine gave a green color with ferric chloride and a urinary amino-acid chromatogram showed a great excess of phenylalanine. Phenylketonuria was diagnosed. He was treated with a low phenylalanine diet. He did not do well but suffered repeated episodes of diarrhoea and dehydration. After four months in hospital he died from pneumonia.

The second son was born in 1958. He has developed normally and does not have phenylketonuria.

Early in 1960, the parents were sent a questionnaire as part of an investigation into the early symptoms of phenylketonuria. They volunteered to come to Toronto and oral phenylalanine load tests were carried out (Table I). The second son was shown to have a normal fasting plasma phenylalanine level (1.3 mg/100 ml.). In discussion with the parents the familial nature of the disease was emphasized and the importance of testing any subsequent sibs for phenylketonuria

*Table I. Phenylalanine Load *Tests on the Parents*

Subject	Age in years	Plasma values in mg/100ml. Fasting Phenylal.	Fasting Tyrosine	1 Hour after Load Phenylal.	1 Hour after Load Tyrosine	Phenylal./Tyrosine Ratio**
Father	44	1.2	1.1	18.9	1.6	13.5
Mother	32	1.0	1.1	12.7	1.8	7.9
10 Normal Subjects	34.1(0.29)	1.29(0.5)	1.51(0.26)	12.1(3.5)	2.8(0.83)	5.0(2.1)
20 Heterozygotes (parents of phenylketonurics)	35.7(6.27)	1.39(0.44)	1.30(0.36)	14.8(3.7)	1.8(0.36)	8.9(1.9)

* O.1 gram of L-phenylalanine/kilo. body weight by mouth after an overnight fast; blood samples taken before and 1 hour after the load.

** Ratio of values at one hour, both expressed in M/100 ml.
Figures in brackets are standard deviations.

Table II. Fasting Plasma Phenylalanine Levels* of the Twins at Different Ages.

Subject	Age	Plasma Phenylalanine (mg/100 ml)	Diet	Subject	Age	Plasma Phenylalanine (mg/100 ml)	Diet
Loreen I	18 days	0.4	Breast fed and usual supplements	Noreen II	18 days	27.0	Breast fed
	24 days	0.9			24 days	32.5	
	6 weeks	0.3			27 days	55.6	Milk formula (Olac)
	6 months	1.2			31 days	73.3	
					6 weeks	0.8	Low phenylalanine (Lofenalac)
					3 months	1.0	
					5 months	0.6	

* Enzymatic method of Undenfriend and Cooper

in the first weeks of life was stressed. A few months later the mother became pregnant and twins were diagnosed. Attempts were made to arrange for cord blood samples and a full examination of the placenta but these were unsuccessful partly because the mother went into labour four weeks before the expected date of delivery.

The Twins

The pregnancy was normal, and the twins were born July 28, 1961. The first baby, Loreen, was delivered as a vertex and weighed 5 lb. 9 oz. (Fig. 2). The second baby, Noreen, was delivered as a breech and weighed 5 lb. 10 oz. (Fig. 2).

Fig. 2. The twins, age 3 months.
Fig. 2a. At this age the facial appearances are quite different. Note that Noreen (left), the phenylketonuric, has a smaller head circumference, an eczematous rash on the cheeks and cracked lips.

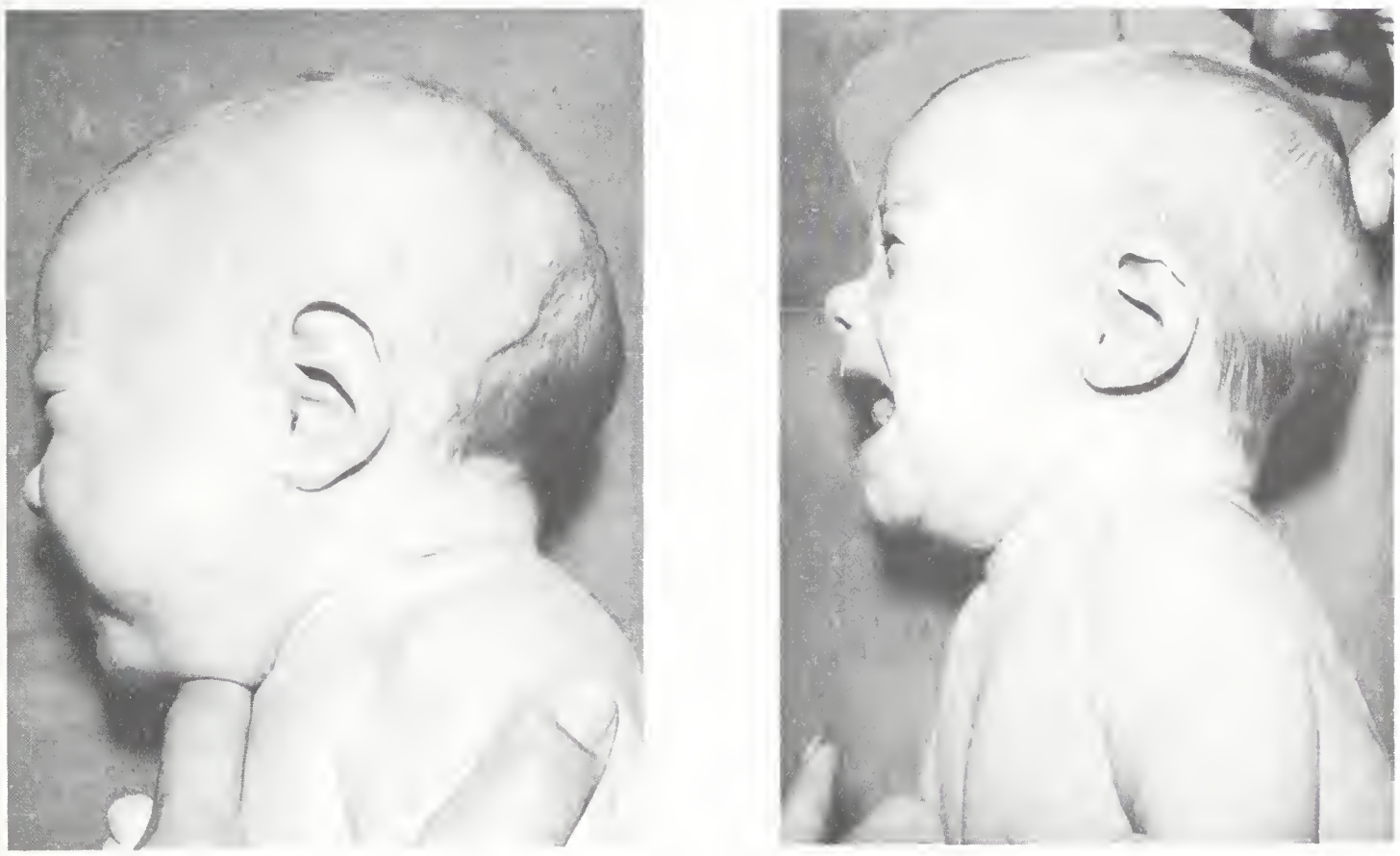

Fig. 2b. Noreen left, Loreen right, showing differences in shape of ears.

Both were girls. There was a single placenta and the mother was told that the twins were identical. There were no difficulties in the early neonatal period and the mother breast fed both babies.

The twins were first seen at the Kingston General Hospital at the age of 18 days. They looked alike, and both had thrived. Neither child's urine showed a color change with 10% ferric chloride. Blood was taken from each child and it was found that the plasma phenylalanine level of the elder twin, Loreen, was normal but that of her co-twin, Noreen, was some twenty times higher (Table II). The twins were recalled and the tests repeated with essentially similar results. Loreen has been found to have normal plasma phenylalanine levels on two further occasions up to the age of 6 months.

Noreen was admitted to hospital. Breast feeding was stopped and she was given a proprietary milk formula (Olac Mead Johnson, Ltd.). Within a few days her fasting plasma phenylalanine level had risen from 32.5 to 73 mg/100 ml. On the 31st day of life her urine became positive to the ferric chloride test for the first time. The next day she was put on to a low phenylalanine diet and has done well on this to date.

The Problem

At this stage the evidence suggested that we had found phenylketonuria in one of monozygotic twins. This seemed unlikely to be true since phenylketonuria is genetically determined by a homozygous recessive gene with complete penetrance. Further investigations were carried out.

The blood groups of the twins were determined by Dr. Howard Steele using 14 different specific antisera; all reactions were the same. Since this evidence did not dispel the possibility of monozygosity, the dermal configurations were analysed as a basis for the determination of zygosity.

Dermal Configurations

A valuable method of determining the zygosity of twins is based on the analysis of the dermal configurations. Dermal prints of the twins, their parents and brother were made by the Hollister and Faurot techniques, and formulated according to standard methods (Cummins & Midlo, 1943). The digital patterns and ridge counts are summarized in Table III, the palmar patterns in Table IV. Discussion of the plantar patterns are here omitted, but tracings of digital, palmar and plantar configurations are shown in Figure 3.

The homolateral percentage differences based on the digital and palmar configurations average 33.0% which agrees with the average differences for dizygotic twins (34.0 ± 2.2) but not with monozygotic (15.9 ± 0.9) (Table V).

An interesting conclusion regarding zygosity can be based merely on the difference between the total digital ridge counts of the twins, which in this case is 40 ridges (Table III). Smith and Penrose (1955) have shown that when the difference in total counts is 33 or more ridges, the relative chances in favor of a dizygotic pair is 33.97.

Analyses of the dermatoglyphics clearly indicate dizygosity.

Ears

The ears of the twins are an anatomical feature which is useful for general identification, but not for critical measurements. Very few genetical studies have been attempted concerning the inheritance of ear-shape, although in monozygotic twins a marked similarity is usually observed. The similarity of the ears of the monozygotic Dionne quintuplets is a case in point.

The ears of Loreen and Noreen are strikingly different (Fig. 26). Noreen's ears show a narrow, band-like helix with a small Darwin's tubercle on each. The antihelix is exposed and somewhat flat. Loreen's ears have a broad fold along the top of the helix, concealing part of the antihelix. The contrasting shapes are excellent distinguishing features for the twins and suggest a dizygotic origin.

Blood Groups

Since the dermatoglyphic analysis gave the first clear evidence in favor of dizygosity, it became important to re-investigate the blood groups of the twins. A total of 30 antisera specific for different antigenic determinants were used to test the parents, the twins and one sib. The twins share the presence or absence of all antigenic determinants tested as shown in Table VI.

From the blood group data the odds in favor of dizygosity were calculated as shown in Table VII. The Lewis system has been excluded; the expected absence of low frequency antigens, such as Wr^a, Mi^a, etc. and the expected presence of high frequency antigens, such as anti-Ve^a, contribute nothing to the odds and are not included.

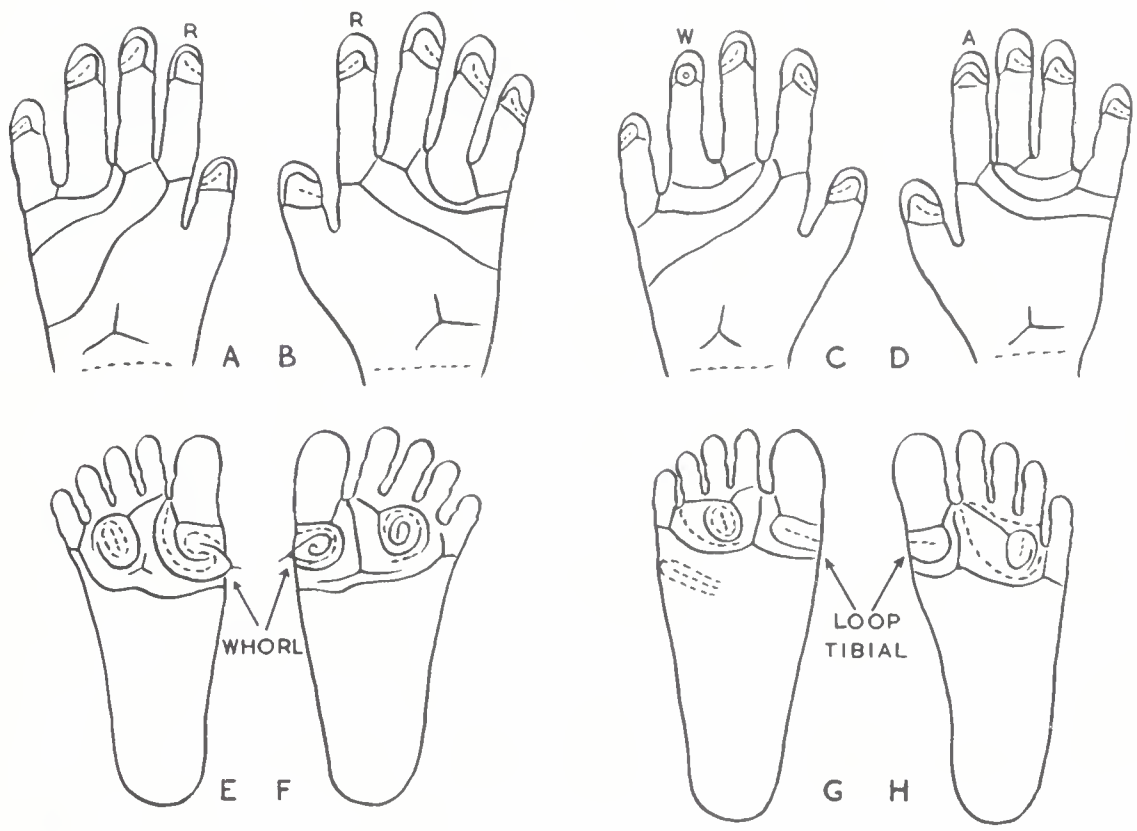

Fig. 3. Outline tracings of palmar and plantar configurations of the twins.

Loreen: left palm (A) and right palm (B); left sole (E) and right sole (F)

Noreen: left palm (C) and right palm (D); left sole (G) and right sole (H)

Table III. Digital Patterns and Ridge Counts of the Twins (Loreen and Noreen), Their Parents and Sib.

Name	Left Fingers 5	4	3	2	1	Ridge Counts L	Total	R	Right Fingers 1	2	3	4	5
Father	U	U	U	R	U				R	R	U	U	U
	0-18	0-18	0-15	16-0	0-16	83	186	103	0-20	0-21	18-0	24-0	20-0
Mother	U	U	U	R	W				W	R	R	U	U
	0-17	0-18	0-17	4-0	5-20	76	137	61	18-10	0-5	0-7	17-0	14-0
Bobby	U	U	U	R	U				W	R	U	U	U
	0-14	0-11	0-17	5-0	0-24	71	140	69	24-14	0-2	14-0	17-0	12-0
Loreen	U	U	U	R	U				U	R	U	U	U
	0-5	0-13	0-10	16-0	0-20	64	118	54	17-0	0-7	10-0	10-0	10-0
Noreen	U	W	U	R	U				U	A	U	U	U
	0-3	3-4	0-11	7-0	0-16	41	78	37	14-0	0-0	8-0	11-0	4-0

Symbols: U, loop ulnar; R, loop radial; W, whorl; A, arch

Table IV. Palmar Formulae of the Twins (Loreen and Noreen W.), Their Parents and Sib

Name	Linear Formula D	C	B	A	Axial Triradii	Hypo	Pattern Formula T/I_1	I_2	I_3	I_4
			Left Palms							
Loreen	9	0	5″	3	t′(17.0)	Au/Ac	0	0	0	0
Noreen	11	0	7	5′	t′(18.0)	Au/Ac	0	0	0	0
Father	9	0	5″	1	t′(15.1)	Au/Ac	V/0	0	0	d
Mother	11	0	7	1	t′(33.6)	TA$_R$	0	0	0	0
Bobby	11	X	7	1	t′(29.0)	Au/Ac	0	0	V1	0
			Right Palms							
Loreen	9	0	5″	3	t(14.0)	Au	0	0	0	0
Noreen	11	0	7	5″	t′(17.0)	Au/Ac	0	0	0	0
Father	11	0	7	3	t′(24.5)	Au/Ac	0	0	0	0
Mother	9	7	5″	3	t′(35.5)	Lr/Lu	0	0	L	0
Bobby	11	0	7	3	t′(32.8)	Au/Ac	0	0	0	0

Table V. Homolateral Percentage Differences in the Digital Patterns and Ridge Counts, Main Lines and Palmar Patterns of the Twins (Loreen and Noreen).

Sets Compared	Digital Patterns	Ridge Counts	Main Lines & Axial Triradii	Palmar Patterns	Average
Loreen - Noreen	20.0	32.0	70.0	10.0	33.0
Monozygotic Twins (53 pairs, Walker 1950)	23.0 ± 1.4	10.9 ± 1.0	17.6 ± 1.4	21.4 ± 1.8	15.9 ± 0.9
Dizygotic Twins (20 pairs, Walker 1950)	47.0 ± 4.4	32.5 ± 4.5	31.5 ± 2.2	32.0 ± 8.9	34.0 ± 2.2

Table VI. Blood Group Phenotypes of W — Family.

	Blood Group System							
	ABO	MNS	P	Rh	Kell	Lewis	Duffy	Kidd
Father	*00*	*NsNs*	P_1	*dce/dce* (rr)	*Kk*	Le (a—b+)	Fy (a+)	Jk (a+)
Mother	A_1	*MSMS*	P_2	*DCe/dce* (R_1r)	*kk*	Le (a—b—)	Fy (a—)	Jk (a+)
Twins	*$A_1$0*	*MSNs*	P_1	*DCe/dce* (R_1r)	*Kk*	Le (a+b—)	*Fy^a Fy^b*	Jk (a+)
Sib	*$A_1$0*	*MSNs*	P_1	*dce/dce (rr)*	*Kk*	Le (a—b+)	*Fy^a Fy^b*	Jk (a+)

Antisera used: Anti-A, -A_1, -B, -H; anti-M(4), -N(3), -S, -s; anti-P_1; anti-D, -C, -Cw, -c, -E, -e; anti-K, -k; anti-Le^a, -Le^b; anti-Fy^a; anti-Jk^a.

In addition, cells from none of the family reacted with anti-Mg, -Vw, -Mi^a, -Wr^a, -ĉe^s, or anti-Kp^a; all reacted, as expected, with anti-Ve^a.

The father was Lu(a—) and Yt(a+); the mother was Kp(b+).

Those phenotypes that are *italicised* may be regarded as representing the genotype.

Unfortunately the Lewis types of the twins must remain in doubt; for the great majority of samples from infants react as Le(a+), the true (adult) red cell phenotypes not appearing for a number of years. This is just one of a number of confusing things about the Lewis system, and occurs because the Lewis antigens are not indigenous to the red cells but are acquired by adsorption of Lewis substances from the serum.

Table VII. Probability of the Zygosity of the Twins (Loreen and Noreen) Based on Blood Groups, Sex and the Frequencies of DZ and MZ Twins in Canada.

Probability of dizygosity:	*Parental and sib data excluded*	*Parental and sib data included*
Twins at birth:	2.5714*	2.57*
Twins being the same sex:	0.5000	0.50
Twins in this family having the same antigens in the:		
ABO system	0.6470	0.84**
MNSs system	0.4556	1.00
P system	0.8489	0.74
Rh system	0.5400	0.50
Kell system	0.5448	0.50
Duffy system	0.8036	0.70
Kidd system	0.8532	1.00
Combined probability pD	0.0648	0.14
Total probability for DZ pD/1+pD	0.0608	0.12
Total probability for MZ 1/1+pD	0.9392	0.88

* Based on Canadian frequencies DZ:MZ = 72.28

** Including the application of Bayes' Theorem (David 1949, p. 94)

The likelihood of dizygosity has been calculated by two methods. The first method uses only the blood types of the twins and gives a total probability for dizygosity of 0.0608. The second and more desirable method includes data of the parents and sibs. These data supply much more information concerning the probabilities of concordance of individual blood types with the result that the total probability for dizygosity is raised to 0.12. The difference between the two estimates demonstrates a pitfall in the Twin Method if only the blood types of the twins are used.

The analysis of the data have shown that by chance alone one would expect complete concordance for all blood types in 14% of such twin pairs even though they are dizygotic. Thus no conclusion is warranted regarding the zygosity of the twins under discussion and "the case rests" on the conclusions to be found in the analyses of dermal patterns and haptoglobins.

Haptoglobins

In addition to the blood systems which are as-

sociated with the red cells, the plasma proteins may also exhibit genetical polymorphism. At least five different plasma proteins are known to exist in more than one chemically distinct form which is genetically determined. The various forms have been recognized principally through applying the techniques of electrophoresis and immunology to plasma proteins.

In 1956 Smithies and Walker showed that individuals could be classified into three groups according to the nature of the protein haptoglobin found in their plasma. These groups were designated 1-1, 2-2, and 2-1, and family studies showed that individuals of the first two types were homozygous for the genes Hp^1 and Hp^2 respectively, while those of the third type were heterozygous. The different types could be recognized by observing the mobility of the haptoglobin zones in starch gel electrophoresis.

A later investigation showed that type.1-1 group could be further subdivided by a different electrophoretic procedure, and it became apparent that there were three alternative forms of the Hp gene, namely Hp^{1F}, Hp^{1S} and Hp^2. Thus altogether there are six possible haptoglobin phenotypes: 1F-1F, 1S-1S, 1F-1S, 2-1F, 2-1S and 2-2. In the Toronto area the frequency of occurrence of these types in the population is approximately 3%, 6%, 8%, 20%, 30% and 33% respectively.

The starch gel electrophoresis pattern on which the preliminary typing of the family was based is presented in Fig. 4a. The patterns of the parents, one the twins (Loreen) and the older sib are all identical (corresponding to haptoglobin type 2-1) while the other twin (Noreen) is distinctly different (haptoglobin type 2-2). The characteristic haptoglobin zones in the type 2-1 serum have moved farther from the origin than those in the type 2-2 serum. The results of the complete study including the newer subclassification of the type 1 gene, are presented in the form of a pedigree in Fig. 4b.

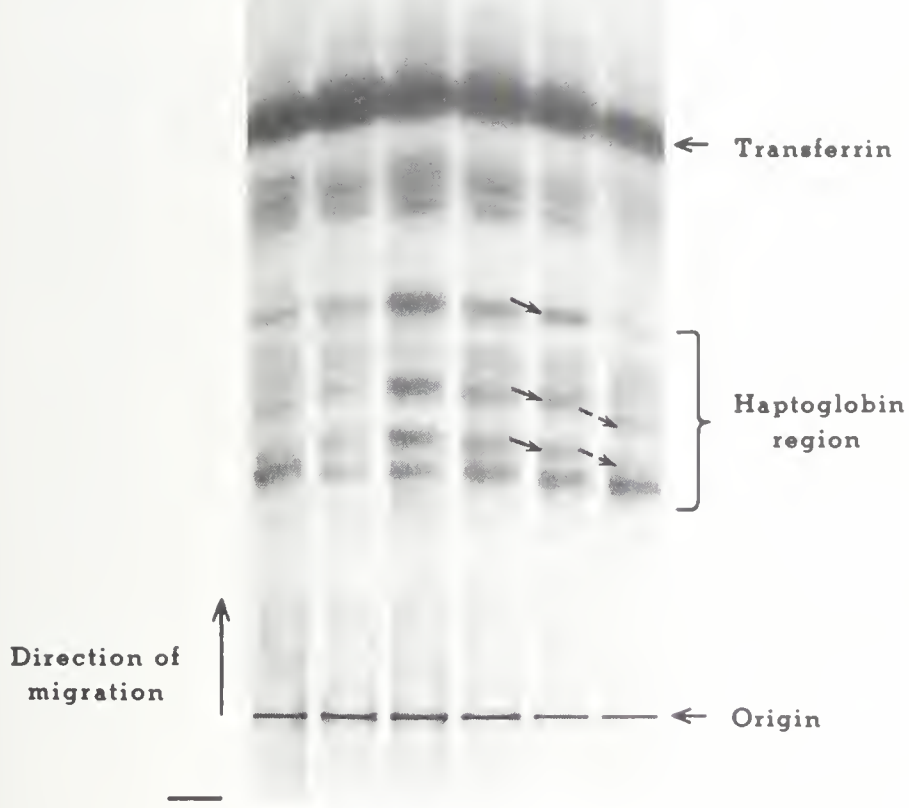

Fig. 4a.

Starch Gel Electrophoresis Patterns with Individual Serum Samples

0.2 ml. of each serum sample was mixed with 0.05 ml. of 3% hemoglobin solution. The starch gel was prepared in 0.021 M sodium borate buffer, pH 8.5. The duration of the run was 18 hrs. at a potential gradient of 6 volts/cm. The albumin zone has migrated out of the gel under these conditions. The characteristic haptoglobin zones in the type 2-1 twin are indicated by solid arrows, in the type 2-2 twin by a dotted arrow.

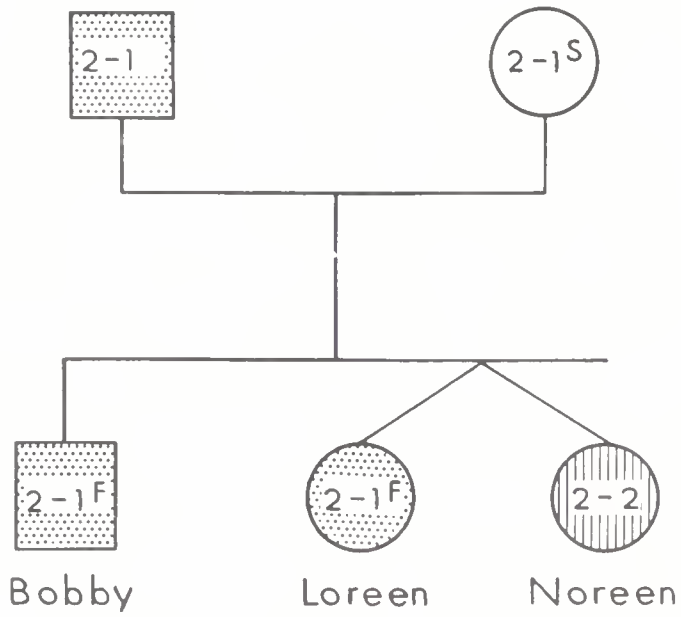

Fig. 4b

Pedigree of W-Family showing Haptoglobin Types.

Another plasma protein, transferrin, also may occur in several forms which are genetically determined. Fig. 4a indicates that all the members of the W-family are identical in transferrin type, probably homozygous type C, which is the only common type in this area.

Discussion

The pitfalls for the unwary in the use of the Twin Method are several and may be briefly summarized as follows:

	MZ	*DZ*
Sex	**Same**	**Same or *different***
Placenta	***Single monochorionic,*** **Single dichorionic or 2 separate placentae**	**Single dichorionic or 2 separate placentae**
Blood Groups	**Same**	**Same or *different***
Haptoglobins	**Same**	**Same or *different***
Dermatoglyphics	**Close similarity**	**Close similarity or marked dissimilarity**

Concordance in sex, blood groups or haptoglobins may occur in either MZ or DZ twins. To be different in any of these features is significant and shows the twins to be DZ.

The placenta may be single and dichorionic or composed of two separate placentae for either MZ or DZ twins. A single monochorionic placenta is the distinctive one and occurs only with MZ twins. A careful examination of the preserved placenta is necessary for documentary evidence. Of little value is a report made from a cursory examination.

Dermal configurations of twins permit a wide variety of comparisons to be made and in certain of these marked dissimilarity indicates with high probability that the twins are DZ.

With complete concordance for a number of blood groups there is a temptation for the unwary to conclude that the twins are monozygotic and to argue that the low probability of this concordance occurring in DZ twins can be disregarded. They will admit that discordance in one or more groups would rule out monozygosity in favor of dizygosity. But they fail to remember that depending on the genotypes of the parents there can by chance be complete discordance for all groups, or various combinations of concordance and discordance of different blood groups, or there may be complete concordance. The latter is no more surprising nor unexpected than other combinations. The fact that Loreen and Noreen showed complete agreement for the presence or absence of all antigenic determinants tested for blood groups does not mean that they originated from one zygote. Indeed the dermatoglyphics and haptoglobins showed otherwise.

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Autoradiography and Irradiation

*by A. G. Bell, M.D., M.A.**

For thirty years it was believed that the human diploid chromosome number was 48. This concept was not challenged until 1956 when Tjio and Levan[1] prepared cultures from several aborted human embryos and utilized special methods of preparation involving the use of colchicine and hypotonic treatment. By these methods many cells were arrested at metaphase with the chromosomes widely spaced, permitting the correct count to be made. This was found to be 46. In the following years numerous laboratories have confirmed 46 as the normal human diploid number, and the tissues examined have included most of the tissues of the body.

It was not the search for the correct count, but the development of the special techniques which led to its discovery that had opened the way to significant and exciting advances concerning the human (and mammalian) chromosomes and about the effects of chromosome abnormalities to the individuals carrying them. These special techniques soon brought to attention the occurence of aneuploidy (or heteroploidy) , that is, abnormal chromosome numbers, in man. The classical case is that of mongolism. In 1959, Lejeune et al[2] found 47 chromosomes in the cells of mongoloid patients. The extra chromosome was one of the smallest, three chromosomes being present instead of two (trisomy) . Other cases of aneuploidy in man found in the early months of these discoveries involved abnormal numbers of the sex chromosomes. The first of these abnormal sex chromosome numbers was observed in some cases of Klinefelter's syndrome[3]. In these cases an XXY sex chromosome complex was exhibited. Some cases of Turner's syndrome were found to have only 45 chromosomes, and an XO sex chromosome complex[4].

Since 1959, many other cases of aneuploidy have been brought to clinical attention. These include the "super-" or "meta-" females who have normal autosomal complements, but sex chromosome complexes of XXX and XXXX. Cases of Klinefelter's syndrome have been found with XXXY, XXYY, and XXXXY complexes. An apparently normal male has been discovered with what is believed to be a XYY complex[5]. Apart from mongolism, abnormalities in autosomal numbers have been discovered in the "Trisomy 13-15 syndrome"[6] and in the "Trisomy 16-18 syndrome"[7].

The tempo of discovery of these simple aneuploid cases has decreased. This is not to suggest that most types of aneuploidy in man have been discovered, indeed many more may yet be found. Most of the conditions depending on chromosome abnormalities now being described, depend not so much on "simple" aneuploidy, but on aberrant chromosome constitution resulting from mosaicisms, translocations and deletions.

A detailed discussion of the chromosome-conditioned diseases is outside the scope of the present writing. Excellent reviews have already been published[8,9].

It is apparent that the frontiers of our knowledge of chromosome-conditioned diseases have been pushed back, but there are still large gaps in our understanding of their etiology. We may speak of non-disjunction and chromosome breaks as etiological factors, but we do not know what causes the non-disjunction or the breaks. For instance, the aging ovary appears to be pre-disposed to non-disjunction, but we do not know why.

The above discoveries, of necessity, have been based on observation, not on experiment. The synthesis of these observations have led to numerous hypotheses, which will have to be experimentally tested. Only in this way will the behaviour of the chromosomes under natural (and experimenal) conditions be understood, leading us to methods whereby we may be able to prevent occurence of such diseases. Such a state of knowledge need not be expected for many years.

Special tools are necessary for such investigations. Two such tools at our disposal are autoradiography and irradiation. These cannot be expected to provide all the answers, but their use will add to our expanding knowledge of chromosome behaviour.

Autoradiography

The incorporation of radioactive atoms into the structure of the chromosome enables that chromo-

***Research Fellow, Department of Genetics, Hospital for Sick Children, Toronto.**

some to write its own "autograph" on photographic emulsion.

Selective labelling of some component of the chromosome is required for autoradiography. In 1951 it had been demonstrated by the use of heavy nitrogen (N^{15}) labelling, that thymidine was a precursor of DNA, the information-carrying code of the chromosome, and that it was not involved in the synthesis of RNA, which is the template upon which the DNA inscribes its instructions to the cell[10]. Since thymidine is specific for DNA, and hence for the chromosome itself, it is the obvious choice for the "label carrier".

The use of most radioactive labels was impractical in chromosome autoradiography. The range of the beta particles emitted from such labels as C^{14} is very large owing to high initial energy, making it impossible to determine the source of such particles. A metophorical equivalent would be trying to write one's name on the head of a pin with a brush used to paint the barn. What was needed was a very fine pen. This pen was provided by tritium, a radioactive isotope of hydrogen (mass three). The beta particles emitted from this source have a very small maximum energy, with a range of one micron, ideally suited to the dimension of the chromosomes.

Taylor et al[11] were the first to utilize tritium-labelled thymidine, and have described their techniques. Briefly, tritium-labelled thymidine is added to a cell culture for a pre-determined length of time (one-half to two hours). The cells that are undergoing DNA synthesis incorporate this tritiated thymidine into the new DNA. Following this exposure, and after removal of the excess label from the medium, the distribution of the incorporated label can be traced after short or long periods. For examination of the distribution of the label at metaphase, the cells are fixed, and slides prepared. The slides are then coated with a thin film of photographic emulsion, and allowed to stand undisturbed for about two weeks. During this time, the escaping beta particles strike the photographic emulsion, and after development a deposit of silver remains over the chromosome from which the beta particle was emitted.

Figure 1 illustrates the dynamic behaviour of the chomosomes and the distribution of the label over two cell generations following labelling. *Figure* 1.*a* represents the interphase, or "resting" chromosomes. An interphase chromosome can be considered to be a thread composed of two strands, shown in the diagram wound around each other. This shows the analogy to the Watson-Crick model of the DNA molecule but it must be pointed out that numerous such molecules compose the chromosome. When the chromosome starts to re-

Distribution of Tritium - Labelled Thymidine
Over Two Cell Generations

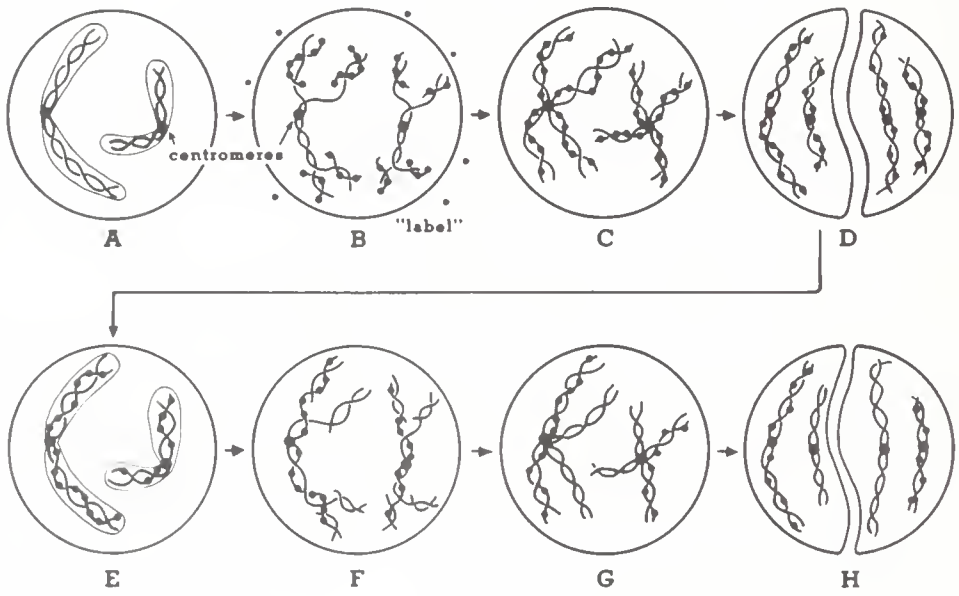

Fig. 1. a) "Resting" chromosomes (interphase)

b) Chromosomes replicating in presence of "label".

c) Metaphase: chromosome strands completely replicated and both chromatids labelled.

d) Daughter cells following cell division.

e) "Resting" chromosomes (interphase) in one of daughter cells.

f) Chromosomes replicating in second cell generation. No label present.

g) Metaphase: chromosome strands completely replicated, only one chromatid of a pair is labelled.

h) Daughter cells of second cell generation. About one-half of the chromosomes in each new daughter cell are labelled. The other half are unlabelled.

plicate, it incorporates into the synthesized DNA, building blocks derived from the cytoplasm. These include thymidine, which we have provided with a tritium label. *Figure* 1.*b* illustrates the beginning of DNA synthesis and chromosome replication in the presence of the tritium label. The chromosome strands "unwind" and synthesis commences with the incorporation of label into the newly synthesized strands. *Figure* 1.*c* illustrates metaphase; the chromosomes have completely replicated apart from the centromere. Both chromatids of the metaphase chromosomes are labelled, but only in the newly replicated stands of each. (See also figure 2a, the first metaphase after labelling.) *Figure* 1.*d* shows the two resulting daughter cells, all the chromosomes of which are labelled.

Figure 1.*e* illustrates one of these daughter cells at interphase, prior to its DNA synthesis and chromosome replication. *Figure* 1.*f* illustrates the beginning of DNA synthesis of these chromosomes in the absence of label. As can be seen in *Figure* 1.*g* (metaphase) only one chromatid of each chromosome is labelled. (See also Figure 2b, the second metaphase after labelling.) This results in roughly one half of the chromosomes in the two daughter cells being labelled. (Figure 1b)

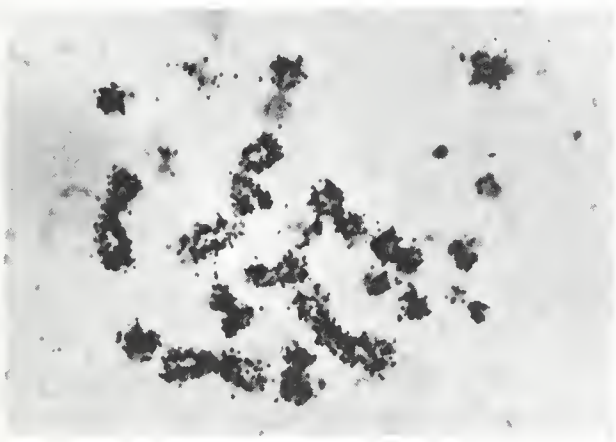

Fig. 2. a

Fig. 2. b

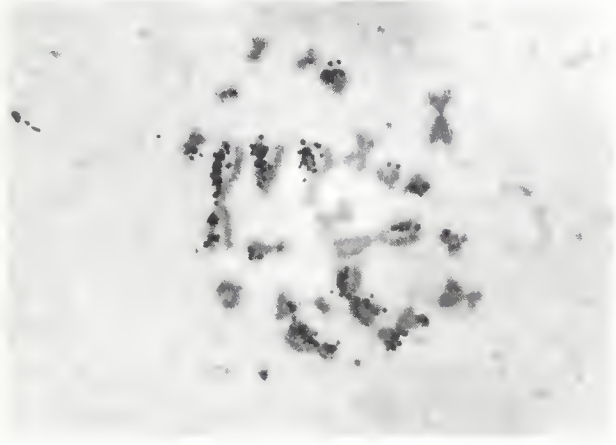

Fig. 2. c

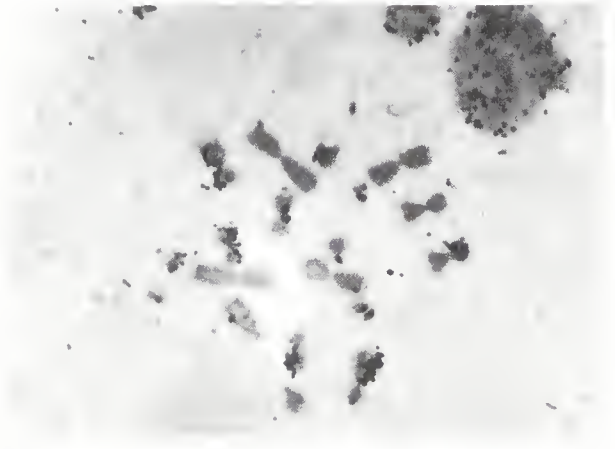

Fig. 2. d

Fig. 2. Metaphase cells (chinese hamster) showing distribution of tritium-labelled thymidine over four cell generations.

a) First Metaphase after labelling. All Chromosomes labelled in both chromatids.

b) Second Metaphase. All chromosomes labelled, but only in one chromatid.

c) Third Metaphase. Only (about) one-half of chromosomes labelled (in one chromatid).

d) Fourth Metaphase. About one-quarter of chromosomes labelled.

Photographs courtesy of M. A. Bender and D. M. Prescott, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Figure 2 contains photographs of actual metaphases after repeated cell divisions following labelling. These cells are from cultures of tissues of the chinese hamster (chromosome number 22). It can be seen that in the third metaphase after labelling, one-half of the chromosomes at metaphase are labelled (but only in one chromatid); at the fourth metaphase, only about a quarter are so labelled.

The above description depicts chromosome behaviour as indicated by labelling studies, that is, the chromosome *behaves* as if it were composed of two strands. Electron microscopic studies[12] suggest that there may be more than two strands and that each strand is composed of individual fibres. This does not invalidate the above, but merely shows that chromosome replication is an extremely complicated process.

Irradiation

The injuries suffered by the pioneer investigators of the ionizing radiations directed attention to biological effects, and out of this developed the work of Muller[13] who made the important discovery that X-rays were capable of inducing mutations. Furthemore he was the first to apply quantitative measurements to these effects[14]. Investigators of these aspects of radiation increased in number so that the literature fast became voluminous as numerous aspects of radiation effects were examined. The political and economic considerations of atomic energy have provided additional impetus to research on the biological effects of radiation.

This discussion is confined to the uses of irradiation in cytogenetic research, and no reference will be made to other areas of research. The earliest observations on chromosome aberrations were made by Muller[13, 14]. In the course of his studies he was able to identify rearrangements of gene order (inversions, translocations and duplications) by genetic evidence, that is, by cross-over effects. His cytogenetic observations on the salivary gland chromosomes of *drosophila* provided support for genetic evidence of chromosome rearrangements and provided verification for the physical validity of linkage maps.

Extensive and pertinent information has been obtained from irradiation experiments on plant material, especially our knowledge of chromosome aberrations[15,16].

Structural Changes in the Chromosomes

Three alternative fates are presented to the chromosome which has been broken by irradiation (or by a radio-mimetic substance). 1) It may remain broken, and the break recognized by the presence of an acentromeric (or acentric) fragment. 2) The broken ends can undergo "restitution", that is, the broken ends heal together effacing the break. It has been estimated that 91% of all irradiation-induced breaks restitute[17]. Some workers feel that the percentage of restitution is even higher, perhaps 99%[18]. 3) Broken ends may unite with others caused by different "hits". This results in the formation of 'two-hit aberrations", and the process is known as "recombination" which competes with process of "restitution".

Types of Aberrations

One-Hit Aberrations. When a break occurs in a chromosome before it has replicated and no restitution or recombination occurs, both the centric and acentric portions replicate. Following replication of the centromere this results in two centric and two acentric fragments at anaphase. The centric fragments behave normally at anaphase, but the acentric fragments may be lost since they have no centromeres to direct them during the anaphase movements.

After chromosome replication a single hit results in the breaking of only one of the chromatids. At anaphase the unbroken chromatic yields an unbroken chromosome, and the broken chromatid yields one centric and one acentric fragment.

Two-Hit Aberrations. Prior to replication, recombination of broken ends resulting from different breaks of the same or different chromosomes results in the formation of dicentrics, tricentrics and rings. Other two-hit aberrations that may be recognized are the results of reciprocal translocations yielding "chromosomes" unlike those of the form under consideration.

More complicated aberrations occur following recombination of the chromatid breaks (after chromosome replication). These are called chromatid exchanges.

Recent Applications

Considerable work has been done in recent years on the effects of irradiation of chromosomes of mammalian solid tissue cultures[19,20]. With such material, classification of aberrations is much more difficult than when simpler forms with fewer chromosomes are used. The methods of scoring of the aberrations is very important. Unfortunately different methods have been used by different workers, and it is difficult to make direct comparisons of results. Also improper methods of scoring have in the past led to erroneous conclusions. Eventually a standardized method of scoring aberrations in mammalian material will be necessary, with the pitfalls clearly indicated.

Experiments were undertaken recently in this centre to determine irradiation effects on the leukocytes of human blood in culture[21]. It is intended that this work represent a pilot study on which more elegant experiments can be based. Certain conclusions can however be drawn from the results, aside from the information obtained which will result in better design of future experiments.

The Culture of Leukocytes

For the past several years it has been possible

to culture leukocytes from normal human peripheral blood. These cultures extended from the success of cultures of bone marrow and of the peripheral blood of leukemic patients. The discovery that the red cell agglutinin, phytohemagglutinin (Difco) increased enormously the number of mitoses in leukocyte cultures along with improved methods of fixing and mounting provided workers with an ideal material for cytogenetic investigation and clinical cytodiagnosis.

The Experiments

These experiments were planned to investgate the production of chromosome aberrations in leukocytes by X-irradiation using different courses of irradiation. The effects of dose, dose-rate and time of irradiation were the main concern of the investigations. In addition these preparations offered an opportunity to investigate the mechanism of polyploid induction. These results may be the most important obtained from these experiments. Polyploid induction cannot be considered a chromosome aberration, but it is an aspect of chromosome behaviour under certain conditions of irradiation.

Bender and Prescott[22], using tritium-labelled thymidine have provided evidence that more than one cell generation takes place in leukocyte cultures harvested after 72 hours in culture, and that at 100 hours, the time when all samples in the experiments under discussion were harvested, most metaphases present were second and third generation metaphases.

Two leukocyte cultures were divided into five samples, and doses of 0, 25, 50, 100, and 200 r of hard X-rays were delivered to the different samples in each culture. The samples in the first culture received their doses of irradiation at 160 r per minute, those in the second at 1.6 r per minute. This represents a 100-fold difference in intensity. The results from both cultures indicated, in the case of one-hit aberrations, the expected linear response to dose, which persisted even after one or two cell divisions. The expected power-function relationship in the case of two-hit aberrations was not observed. This expected power-function relationship is due to the fact that a two-hit aberration depends on two independant breaks occurring and interacting, and thus their incidence should vary with the square of the dose. That this was not observed in these experiments was most likely due to small sample sizes.

There was a definite effect of dose-rate on the production of two-hit aberrations, much fewer were produced at the lower dose rate. This was expected since fewer breaks are produced per unit time at low intensities which can interact with each other, so restitution is favoured over recombination. The data on the one-hit aberrations have not been completely analysed, but so far they suggest that there was no difference in the number of one-hit aberrations at the different intensities. This too was expected.

Another experiment investigated the effects of time in culture of irradiation. Five samples were given 259 r at 207 r per minute at different times in culture. The times selected were 24, 48, 72, 96 and 100 hours after culture preparation. These samples will be referred to as T1, T2, T3, T4 and T5. T5 provided no numerical data. T4 was the only sample to contain chromatid aberrations. This sample was harvested 4 hours after irradiation, thus all metaphase cells in this sample had duplicated chromosomes at the time of irradiation.

The incidences of aberrations in T1, T2, and T3 were not significantly different from each other, suggesting that the first one or two divisions after irradiation had little or no effect on the numbers of aberrations present. This was observed for both one-hit and two-hit aberrations. Acentric fragments tend to be lost at mitosis, therefore, the early irradiated samples were expected to show fewer one-hit aberrations than those irradiated later. Similarly such two-hit aberrations as dicentrics, tricentric and rings were expected to result in cell suicide at mitosis, therefore fewer of these aberrations were expected in the early irradiated sample.

Induction of Polyploidy

A most interesting finding, and one not compromised by small sample size, was the increased incidence of polyploidy in two of the T samples. In addition there were two types of polyploids observed. The one form was the typical tetraploid cell, where the chromosome number is doubled (Figure 3a). The other form was the diplochromosome cell. Here the chromosomes have four strands at metaphase, a result of "endoreduplication". (Figure 3b) Diplochromosomes are induced by "regression" of the interphase chromosome during or after replication to an earlier stage of interphase. This regression occurs spontaneously (rarely) but has been observed in other irradiated materials. After the regression is overcome, the chromosomes again proceed to mitosis. This

Fig. 3. a

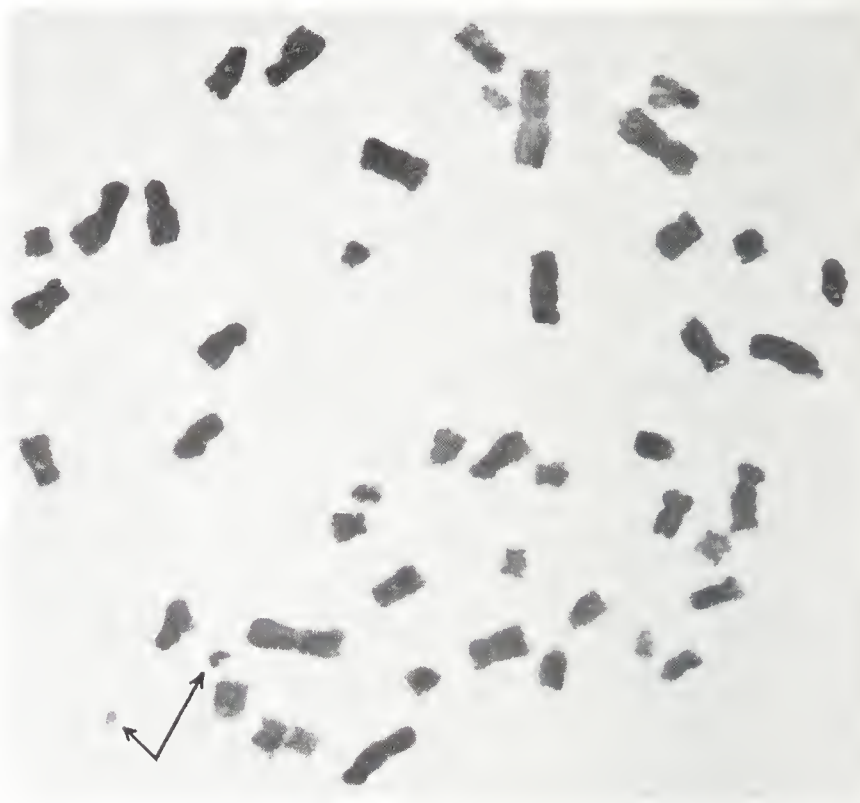

Fig. 3. b

Fig. 3. Polyploid cells.
a) Typical tetraploid cell.
b) Diplochromosome cell (endoreduplication). Arrows indicate two halves of a diplochromosome which have separated.

involves a further replication, resulting in the fourt-stranded diplochromosome seen at metaphase in these cells.

There was no increase in the incidence of polyploidy in the dose and dose-rate experiments. These samples had all been irradiated 16 hours after culture preparation. This supports previous work[22,23] indicating that initiation of mitosis (and hence chromosome replication) requires a certain interval of time in these leukocyte cultures). T1, irradiated 24 hours after culture preparation, contained 103 polyploid cells per thousand (10%). In these, the tetraploid cells outnumbered the diplochromosome cells 73 to 30. In T2, irradiated at 48 hours, 79 of 1000 metaphase cells (8%) were polyploid. In this sample, the ratio of the two types were reversed; 52 dioplochromosome cells to 27 tetraploids. T3 and T4, irradiated at 72 and 96 hours showed polyploid incidences no different from the controls. The changing ratios in T1 and T2 suggests a transition of the irradiation-induced displochromosome cells to tetraploids, this transition requiring time and probably an uninterrupted mitosis. This extra time was the advantage afforded T1.

These experiments suggest a method for inducing endoreduplication at will, at least in leukocycte cultures. As mentioned earlier, it has been known for some time that irradiation induces diplochromosomes but their deliberate production in large numbers and quantitative analysis of these have previously eluded the investigator. The application of labelling techniques to irradiation-induced endoreduplication may in future provide interesting results that will help elucidate other aspects of chromosome behaviour.

Conclusion

Clinical and cytogenetic observations have brought to attention the presence in man of disease entities which are due to abnormalities in chromosome structure and behaviour. Considerable research is necessary in order to examine the hypotheses based on these observations. The techniques of autoradiography and irradiation in conjunction with modern mammalian tissue culture techniques will be of increasing importance in our search for understanding of the problems involved.

In addition, extension of this type of work may

help our understanding of the etiology of those forms of cancer whose cells contain hypotetraploid sets of chromosomes[24]. In these cells an original tetraploidy possibly due to endoreduplication must be assumed to have existed.

Similarly, the study of irradiation-induced chromosome aberrations *in vitro,* and standardization of the results may provide measuring-sticks which will assist assessment of dose received by accidentally irradiated patients.

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Book Review

Medical Pharmacology, by Andres Goth M.D., C. V. Mosby Co., 551 pages, 1961, $11.55.

To the medical student and practising physician, the selection of an acceptable pharmacology text can be a problem. The book must be concise, yet not to the point of dryness. It must contain contemporary ideas, but not at the expense of the older, established concepts.

In this regard, Andres Goth has succeeded admirably, for his text is both enjoying and enlightening. Pharmacology can certainly be a bore, for the odd Latin, chemical and trade names of various drugs are hardly challenging, and less so are the various doses ascribed to them. Goth conquers this problem by "emphasizing principles and concepts, rather than the type of practical information which students memorize and promptly forget."

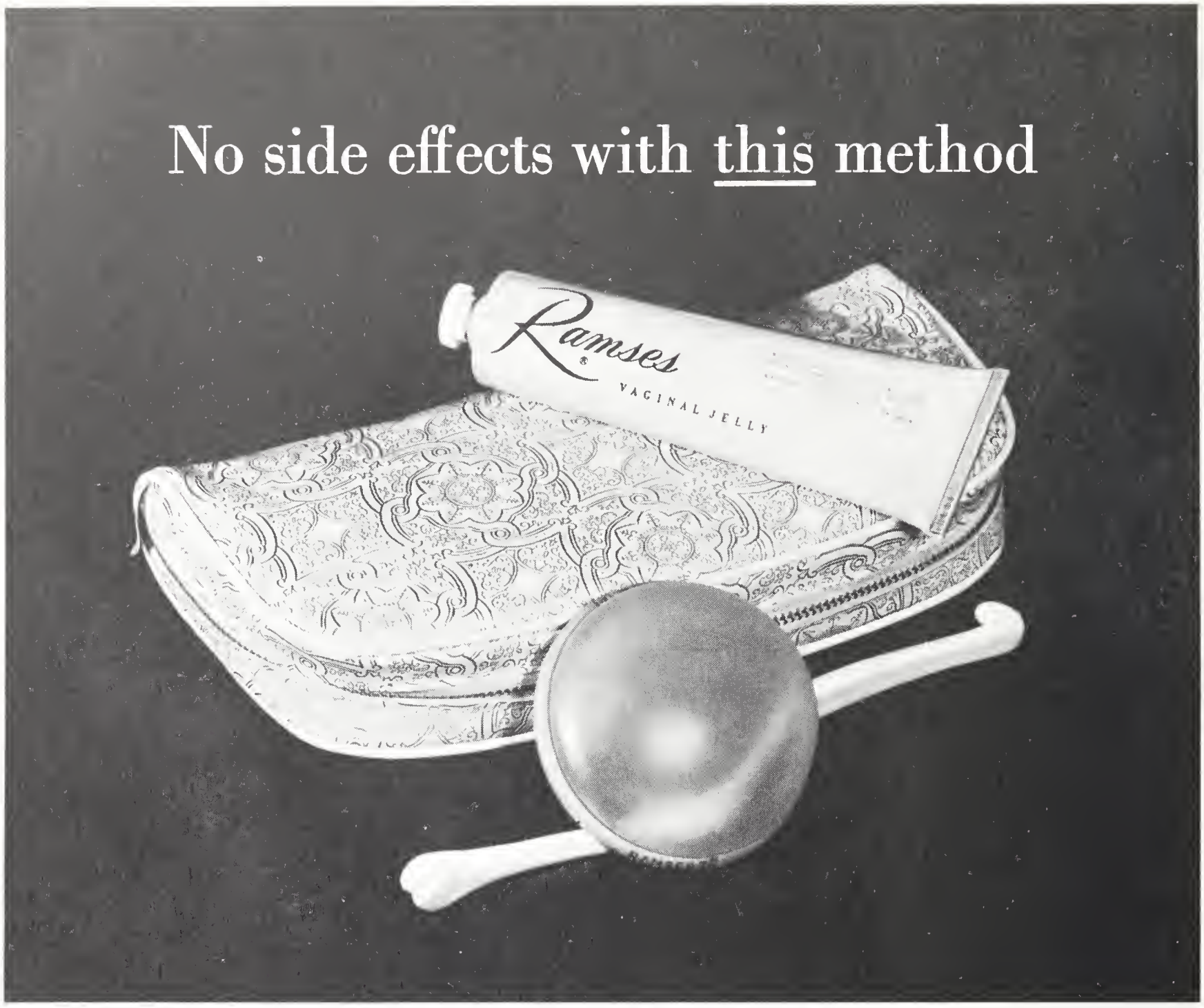
The text is a model of organization, drugs being classified with regard to function, for example, there is a section on anesthetics, and one on chemotherapy etc. Moreover, within each main section the various drugs are sub-classified. For example, drugs ordered in cardiovascular disease are subclassified into the digitalis glycosides, antifibrillatory drugs, coronary vasodilator drugs, anticoagulant drugs, and diuretic drugs.

The development, concepts, theories and the various drugs are then discussed in a lucid, stimulating manner from a medical viewpoint. Particularly excellent, is the section on neuropharmacology, which is not covered in such an intriguing and encomparising manner in any current pharmacology text. This section includes a discussion of psycho-pharmacology, with appropriate reference to the tranquilizers, a subject which should be foremost in the physicians' mind.

If what is desired are the essential facts about important drugs, without repetitious discussion of compounds which have little practical or theoretical importance, and an absorbing and rewarding reading experience, this is the book! (Moreover, it is the least expensive of any of the recommended texts) .

F.L.

No side effects with <u>this</u> method



Patients are unwilling knowingly to risk side effects, even "mild" ones. Physicians are loath to risk any basic alteration of female physiology. Both value *continued good health and well-being* above what is merely new, particularly for the young mother. Busy rebuilding strength after having come safely through childbirth, she wants a method of conception control sure to keep her feeling at her best until she plans for her next baby.

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*Tietze, C.: Proceedings, Third International Conference Planned Parenthood, 1953.

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Risk to Man from Radiation

*by Diane Wilson Cox, B.Sc. M.A.**

With the advent of the "atomic age," there has arisen a great interest, on the part of both scientists and laymen, in possible consequences from exposure to ionizing radiation. Recently, the possible effects of fallout have elicited numerous speculations, claims and fears. In this article, basic findings in experimental radiation studies will be reviewed. The investigations of human populations exposed to radiation will then be presented and assessed.

Two types of effects from ionizing radiation are now recognized: somatic effects are expressed only in the exposed individual; genetic effects alter the basic hereditary material and are expressed, not in the exposed individual, but in subsequent generations. The latter effects are therefore a problem of whole populations, not only of those exposed. Genetic effects have been the main concern, to date, where small doses of radiation are involved.

Studies with Experimental Animals

Muller demonstrated conclusively in 1927 that high doses of ionizing radiation produce "mutations" in the germ cells of living organisms.[1] Such mutations, or changes in the genetic material, may be passed on through many generations before elimination. All kinds of ionizing radiations (e.g. gamma rays, x-rays) which have been tested on living animals have been shown to produce mutations. Mutations include in the broadest sense, structural changes of the chromosomes, primarily associated with high doses, as well as point mutations, usually considered to be changes of single genes.

Mutations have occurred continuously throughout time and have made evolution possible. The causes of such naturally occuring or "spontaneous" mutations are not fully understood, although background radiation surely makes a contribution. In experimental studies, spontaneous and induced mutations appear to be qualitatively similar.

Results of numerous animal experiments have shown that the frequency of mutations increases linearly with dose. This relation has been shown to be valid down to 25 roentgens (r.) for lethal mutations in the fruit fly *Drosophila*[2], an organism often used in genetic studies. At lower doses, large numbers of experimental organisms are required in order to detect small changes in the frequency of mutations. However, results from these low doses are of greatest concern to man. No threshold has yet been found for the production of mutations: any dose can cause mutations.

Estimates of the genetic hazard to man have been based on mutation rates found in *Drosophila* and the mouse. Since many assumptions must be made in the transference of estimates from other organisms to man, present estimates are somewhat speculative and uncertain.

Studies in Human Populations

Studies in man have been undertaken in an attempt to provide more meaningful results.

No group of individuals has been exposed to an amount of radiation sufficiently large that on surveying the offspring of the irradiated parents, an increase in the occurrence of a specific mutant phenotype could be expected[3]. In studies of *Drosophila,* it has been shown that thousands of progeny and elaborate breeding methods are required in order to detect the occurrence of mutations. The approach for man must be, of necessity, much less specific, involving numerous vital statistics of the progeny.

Certain of these vital statistics are possible indicators of genetic damage. However, each indicator is influenced by numerous other factors; no unique yardsticks of genetic damage have yet been found. It is therefore important that, as far as possible, irradiated and control series should differ only in radiation history.

Sex Ratio

The number of males per 100 females, that is the sex ratio, among offspring of exposed individuals, may be a measure of genetic damage. Lethal mutations, leading to the early death of an organism may be dominant or recessive to their normal alleles. The theory behind the use of the sex ratio as an indicator of genetic damage is shown in figure 1.

If mothers were irradiated, induced X-linked

Department of Genetics, Hospital for Sick Children

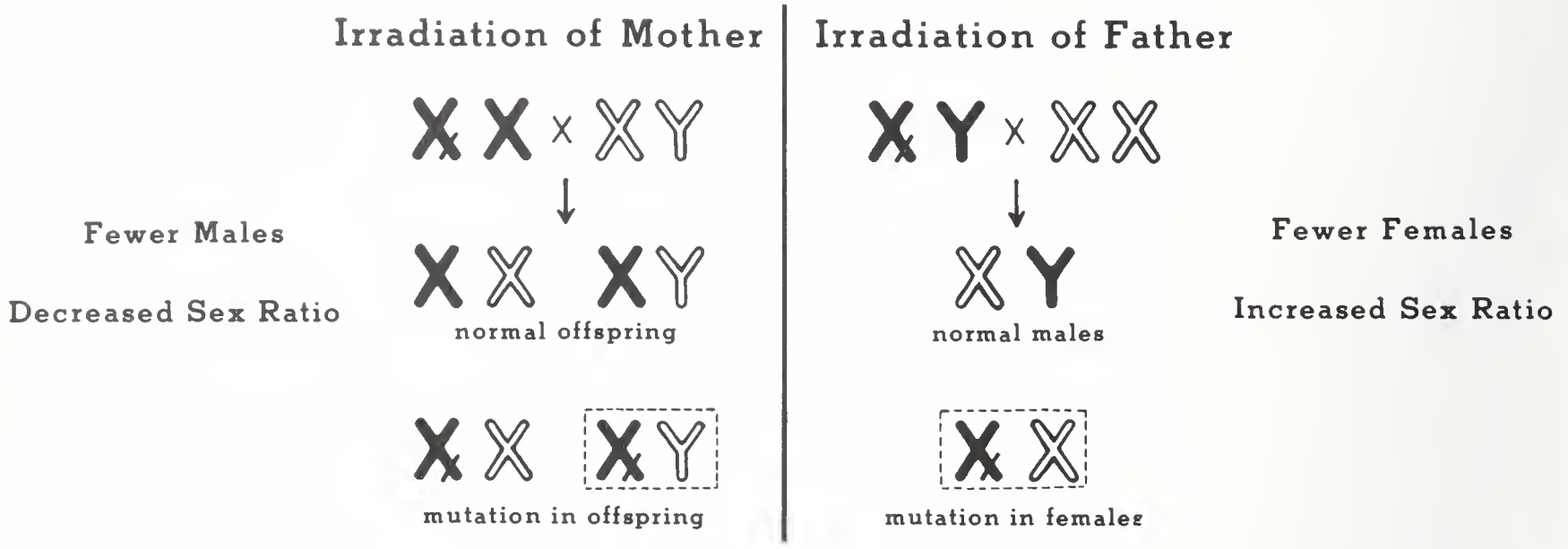

Figure 1. The sex ratio as a possible indicator of genetic damage.
barred X: mutation on X-chromosome.
outlined X or Y: sex chromosomes from non-exposed mate.

dominant lethals would similarly reduce both male and female births. X-linked recessive lethals would be expressed most frequently in male offspring since a corresponding allele is lacking on the Y chromosome. There would be a decrease in the number of male births and a corresponding decrease in the sex ratio.

If fathers were exposed, induced X-linked lethal mutations could be transmitted only to female offspring. The dominant lethals would decrease the frequency of female births, thus increasing the sex ratio.

The sex ratio is influenced by several factors which must be considered in studies of radiation effects. The sex ratio shows a significant decrease with increasing paternal age and a significant but smaller decrease with increasing maternal age[4]. The sex ratio may decrease with increasing birth order, although the age factor may be responsible[5].

Frequency of Fetal Deaths

In animal experiments, autosomal lethals are the largest group of induced mutations which produce effects detectable in the first generation of offspring. The majority of the lethals detected would be dominant, although a recessive lethal would act if the affected individual happened to have a similar mutant allele of spontaneous origin. Lethal mutations might cause an increase in the frequency of fetal deaths among the offspring of exposed parents.

Frequency of Infant Deaths

Lethal or semi-lethal dominant mutations induced by radiation could produce a condition incompatible with infant survival, as outlined for stillbirths. The induction of recessive detrimental mutations could produce a less obvious effect which would nevertheless make the affected individual more susceptible to death in early years.

Frequency of Malformation

Visible effects of mutations may be detected by an increased frequency of malformations in the offspring of exposed parents. Some malformations may be recognizable at birth, while others, although congenital, may not become evident until later.

The frequency of congenital malformations is one of the most commonly used indicators of genetic damage, although the limitations and uncertainties in its use have sometimes been overlooked.

It is difficult to determine what to classify as a malformation: no sharp dividing line exists between normal and abnormal or between a major and minor malformation. When the criteria have been established, the irradiated and control groups must be treated similarly.

The ideal situation would be to confine the analysis to malformations with a genetic cause. However some malformations are entirely genetic in origin, some have a known genetic component, and some, appearing to be non-genetic, may have a genetic factor unrecognized as yet. Because of the problems involved in classification, malformations of any type have usually been considered.

The frequency of congenital malformations is affected by a variety of factors. There is a significant increase in frequency with increasing birth order and with increasing maternal age[6]. Numerous environmental factors, including maternal diet, hormonal factors and prenatal infection may influence the frequency of congenital malformations.

Birthweight and Anthropometric Data

Mutations producing a detrimental effect in an individual may be reflected in changes in birth weight and certain body measurements (e.g. height, weight, and head size). Radiation-induced genetic changes would depend upon the extent to which these measurements are genetically controlled.

Birth weight and body measurements are affected by many other factors. Birth weight, for example, increases with increasing birth order and decreases slightly with increasing maternal age[7].

Surveys of Exposed Human Populations

The above-mentioned indicators of genetic damage have been investigated in surveys conducted among the offspring of parents who have received significant doses of ionizing radiation in seven different circumstances. Results have not been consistent, probably due in part to the influence of other factors upon the indicators of genetic damage. Further problems and fallacies will be pointed out in the discussion of each study to follow.

Survivors of the Japanese Atomic Bombings

Neel and Schull[8] have presented data on the offspring of these survivors, investigated through the Atomic Bomb Casualty Commission. Over 76,000 offspring of 41,000 parents exposed to radiation from Atomic Bombs in Hiroshima and Nagasaki were studied. Mean doses of total body radiation received were estimated, from the location and shielding of individuals, to be in the order of 5 to 10 r., 50 to 100 r. and 200 to 400 r. (Gonadal doses would be lower).

Sex ratio data came from three sources: in the period 1948 to 1953 all infants were examined by Japanese physicians, and those of related parents were separated from those of unrelated parents; in the period 1954 to 1955 the sex of offspring was reported by the parents, all of whom were unrelated. These groups were further divided according to parental exposure, that is father, mother or both exposed. Effects of paternal and maternal exposure were considered separately in the final category. Within each of the 12 groups, there were at least three different levels of dose. A significant deviation was found in one group, in which mothers were exposed. In 11 of the 12 groups, the direction of the deviation agreed with the assumption that lethal X-linked mutations had been produced. It is unlikely that such a distribution was due to chance.

After the reporting of each birth by a Japanese midwife, the child was examined by a Japanese physician for congenital malformations. Stillbirths, neonatal deaths and abortions were reported. A random sample of infants were re-examined at nine months of age for the collection of data regarding body measurements.

For the statistical analysis of the data, corrections for differences in parental age and birth order were applied. Significant results were found in a few scattered exposure groups, however there was no trend consistent enough to positively demonstrate genetic damage.

The data have recently been re-analysed by de Bellefeuille[9]. Comparisons were made between pairs of groups in which one parent was exposed lightly or heavily, with the other parent unexposed in each case. Statistically significant deviations, suggestive of genetic damage, were found for the sex ratio and frequency of stillbirths when mothers were exposed, and for the frequency of neonatal deaths when the fathers were exposed. It was pointed out that the frequency of malformations is under-reported since congenital heart disease and great vessel disease were not included. Some malformed infants may have been disposed of before examination, thus presenting another source of lost data.

This is the largest study of a radiation-exposed human population undertaken to date. It has not demonstrated drastic effects upon the offspring of even those parents receiving the highest dose.

However, there does appear to be some indication of genetic damage, particularly in the alteration of the sex ratio.

Individuals receiving therapeutic pelvic radiation

The sex ratio among offspring of 570 men and 206 women treated by pelvic radiotherapy has been reported from France[10]. Skin doses in the pelvic region were estimated to be over 1,000 r. The gonadal dose, although not estimated, must have been high since no shielding was used.

The males were divided into two classes depending upon the reason for treatment, although this did not appear to be correlated with the dose received. In each group, the proportion of males born after radiation showed a non-significant increase when compared with the proportion of males born before radiation, in accordance with the assumption of lethal induced mutations. The inclusion of fathers who had children both before and after radiation would have made the amount of increase in the sex ratio less than the true increase. This is due to the cancelling effects of increased age of the father and higher birth orders for children born after irradiation.

The sex ratio of offspring born to women before radiation was compared with that among offspring born after radiation. The sex ratio showed a non-significant decrease after radiation, in agreement with genetic prediction. Parental age and birth order effects in this case accentuate the true decrease.

Although no single comparison was statistically significant the sex ratio among the offspring of each of the three groups showed a deviation in the expected direction.

The data were obtained by questionnaire. Although only 37 per cent of the fathers and 34 per cent of the mothers replied, the sex ratio should not be biased. No other data on the offspring were analysed because of their incompleteness.

Women treated for sterility

A follow-up study was conducted by Kaplan among 671 women who received a mean dose of 65r. to the ovaries during X-ray treatment for sterility[11]. Inquiry was made by mail, by telephone or through the family physician.

The proportion of males was non-significantly decreased among the liveborn offspring of radiated mothers when compared with the general population. This agrees with genetic prediction, assuming that lethal X-linked mutations had occurred.

Data on fetal deaths and congenital malformations are of limited value because of the lack of a control series.

Radiologists

The dose received by the radiologists studied in the United States has not been estimated[12]. There are undoubtedly wide differences between individuals. Information was collected on offspring of 2,236 male radiologists and 1,570 male non-radiological medical specialists.

The proportion of male offspring showed a non-significant decrease among liveborn offspring of radiologists when compared with those of controls. This deviation is in the opposite direction to that expected from the genetic theory. The sex was unknown for 15 per cent of the offspring, however this would not have been expected to introduce a bias. Parental age differences between the two groups were not considered, however effects would probably be small where such large numbers are involved.

The frequency of congenital malformations was higher at each birth order among the offspring of radiologists, exposed throughout marriage, than among the offspring of controls, unexposed throughout marriage. The difference was statistically significant only at the first birth order. Differences could be due to bias in response: the study was conducted by questionnaire with a 74 per cent and 54 per cent response from the radiologists and controls respectively. Those radiologists with malformed offspring might have replied more readily than those with normal offspring. A further source of error may have been introduced by the inclusion of certain minor defects which could have been considered insignificant by some parents.

The fetal death rate showed a non-significant increase among offspring of radiologists over that of controls within each birth order.

X-ray technicians

The offspring of 232 male Japanese X-ray technicians were compared with the offspring of 300 non-exposed pharmacists[13]. The estimated dose received by the technicians was 35r. per year, although the mean period of exposure before the birth of their offspring was not stated.

A significant increase in the proportion of males

was observed among offspring of the technicians, as expected from the genetic theory. The frequency of sterility, and of abortions and stillbirth among offspring were significantly higher for the technicians than for the controls.

The data were obtained by questionnaire. Possible effects from differences in parental ages and birth order of the offspring were not considered.

Individuals receiving fluoroscopy during childhood

No dose was estimated for this group of 746 females and 734 males who received an average of 17 fluoroscopic heart examinations during childhood[14]. The exposed individuals were abnormal since all had heart defects.

The stillbirth frequency among the offspring of the exposed group did not differ significantly from the mean value for the New York State population. The frequency of congenital malformations among offspring was reported by the parents upon questioning or observed during a physical examination. However no control group was used.

Women receiving multiple pelvic X-rays during childhood

The 78 mothers in this study had been treated for congenital dislocation of the hip at the Hospital for Sick Children, Toronto[15]. Diagnostic pelvic X-rays were frequently required during the treatment period. The mean number of pelvic X-rays received during childhood was 37 films per child. The mean ovarian dose, measured in wax phantoms, was a minimum of 10r. This was probably an underestimate due to the loss of records on the number of films used and to the uncertainty surrounding the techniques and equipment used in the 1920 to 1940 period. The true mean dose could have been three or more times higher than that estimated.

The exposed mothers' 135 sibs who had offspring provided a good control series because of the similarity of social, economic and genetic backgrounds of the two groups. There was no significant difference in the distribution of birth orders among the offspring of the two groups. The mean parental age was significantly higher for the controls, probably because of their higher mean age and therefore more complete families at the time of interview.

Information was obtained by personal interview with each of the exposed mothers. Information regarding the control sibs was also obtained in these interviews and was verified by contacting the sibs themselves (28 per cent personally, 61.5 per cent by questionnaire).

The proportion of males was lower among the 201 offspring of the exposed mothers than among the 400 offspring of the controls. This decrease in the sex ratio, although not statistically significant, is in the direction expected if X-linked lethal mutations had occurred. Because parents in the exposed group were younger, the observed decrease in the sex ratio was less than the true decrease.

There was no evidence for detectable genetic damage as expressed in increased frequencies of stillbirths, neonatal deaths, or abortions and miscarriages.

The mean birth weight of male offspring was significantly lower among offspring of the exposed mothers than the controls. The difference between mean paternal ages would tend to reduce the true difference. However, reporting of the birth weights was incomplete (95 per cent and 72 per cent for offspring of exposed mothers and controls respectively) and may have biased the results. There was no significant difference between the mean birth weights of the female offspring of the two groups.

Those congenital abnormalities of the offspring which were reported by the parents and required medical care were recorded. The frequency of all abnormal offspring born to the exposed mothers was significantly higher ($P=0.006$) than the frequency of such offspring born to the control sibs. When only those abnormalities requiring hospitalization were considered, the difference was still statistically significant ($P=0.008$).

The possibility does exist that women affected with congenital dislocation of the hip are in some way constitutionally abnormal and that their offspring would show the observed differences even if no radiation had been involved.

Evaluation of the Hazard to Man

In the investigations of the offspring of individuals exposed to ionizing radiation, alteration of the sex ratio appears to be the most significant finding. Although few of these deviations are statistically significant, the direction of the deviation is in the predicted direction among the offspring of 17 exposed groups and in the opposite direction in only two. It therefore seems probable that X-linked lethal mutations are indicated by a

decrease in the sex ratio when mothers receive radiation and an increase when fathers receive radiation. An increased frequency of congenital malformations may have been detected among the offspring of exposed individuals, however this has not been a consistent finding. Other possible indicators of genetic damage have not shown consistent or definite positive results.

The problems in the use of possible indicators of genetic damage have been pointed out. Even negative results cannot be assumed to indicate lack of genetic damage. Only a small amount of the total damage would be expected to appear in the first generation offspring. There is reason to believe that radiation, in any quantity, will produce mutations in man as in other animals and that such mutations are usually detrimental. Studies in man indicate at least that no extensive detectable damage is produced in the first generation offspring as a result of the level of dose to which groups of individuals have been exposed. However, the important question still unanswered is how much mutation the human race can withstand.

It is of interest to note the amount of radiation which individuals are receiving at the present time. Doses are estimated for a 30-year period, which on the average extends into the mid-reproductive period of an individual. This is considered to be the mean genetically-important dose. The estimated 30-year doses from all sources are shown below, computed from world wide averages[16].

Natural sources	3 r.
Medical & dental sources	0.5-5 r.
Occupational exposure	0.06 r.
Fallout	0.06 r.

At a time when there is such a great concern over fallout from nuclear weapons testing, it may be surprising to see its magnitude in comparison with that from other sources. The figure assumes cessation of testing at the end of 1961 and is, of course, only an estimate.

Medical and dental X-rays are the largest source of man-made radiation at present. It has been estimated that 75 to 90 per cent of the total dose from medical use is from diagnostic X-rays[16]. This now seems to be the most fruitful area in which to strive for the reduction of radiation exposure.

The benefits of the X-ray as a diagnostic tool cannot be denied. There is no need to hesitate to use X-ray examinations that are deemed necessary for the welfare of an individual. There is however, a definite need to make sure that the benefits of an X-ray outweigh the risk envolved. There is an even greater need to fully utilize the modern techniques for dose reduction, such as filters, coning devices, fast screens and films, and lead shielding. Precautions are necessary particularly in examinations of the pelvis or abdomen in which the gonads may be in the direct beam of exposure.

Until we know just how much radiation human populations can safely tolerate, caution must be exercised, not only for the sake of the exposed individuals, but for present and future populations which may inherit the damaged genes.

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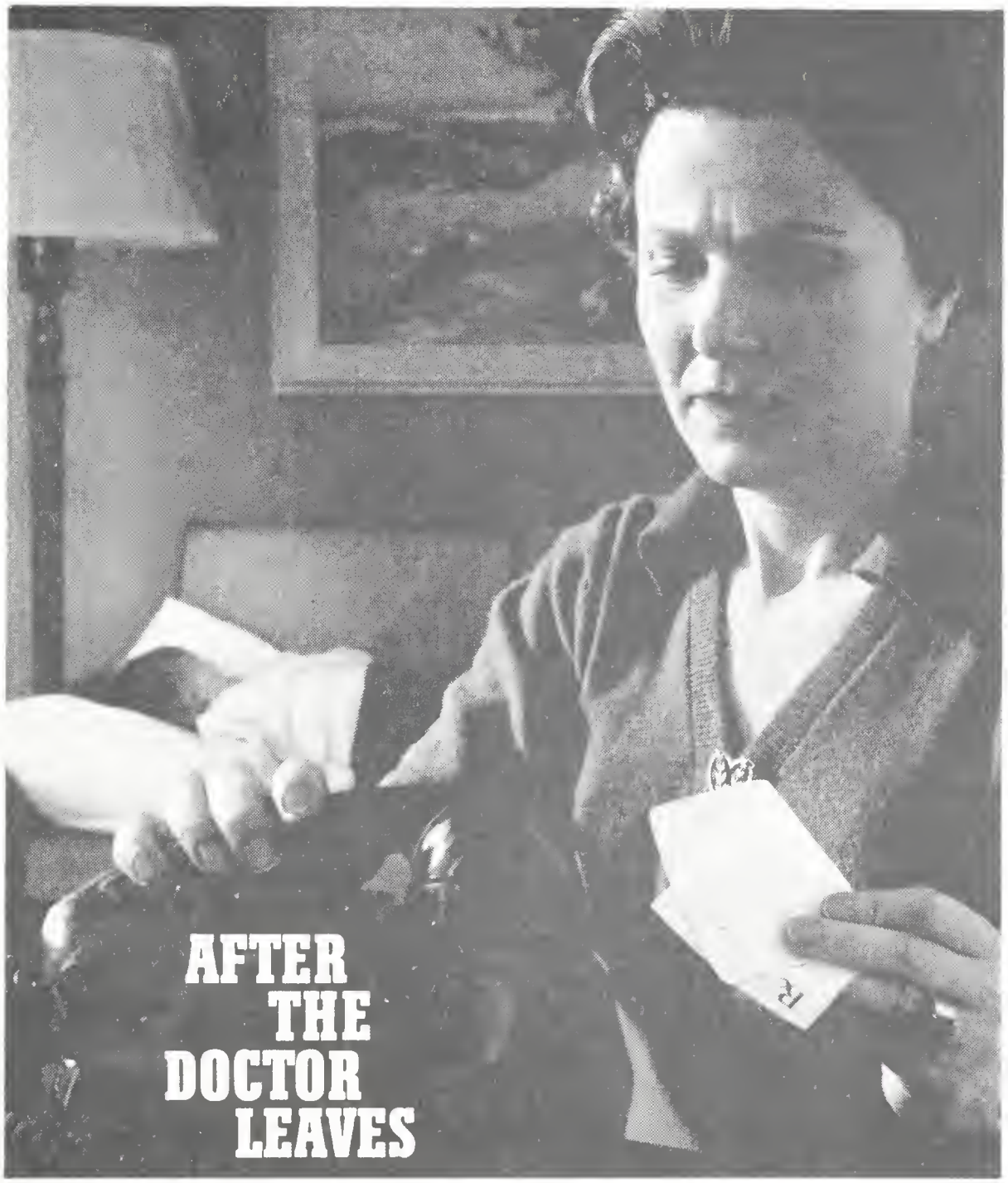

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UPJOHN ABSTRACT: *Prevalence of Amoebiasis in the United States and Canada,* Burrows, Robert B., Amer. J. Trop. Med. 10:172-184 (March) 1961.

The prevalence of amoebiasis caused by Entamoeba histolytica in the United States and Canada was determined by collecting data from all papers which reported surveys and were published in 1945 or later; 166 surveys on nearly a quarter of a million persons were used. It was found that approximately 1% of the population of Alaska and Canada may be infected, about 2 to 4% of the population of northern United States may harbor the infection, and the prevalence in southern United States may not be more than 4 to 6%.

These figures on the prevalence of amoebiasis in the United States are much smaller than previously published estimates of 10 to 20%, which were much too high because of the inclusion of E. hartmanni and because the majority of surveys were carried out on groups expected to show a high prevalence of amoebiasis.

A more accurate picture of the prevalence of amoebiasis in various sections of the United States can be obtained only if many more surveys are conducted, and the author made some suggestions regarding procedures for future surveys. He suggested that (1) more surveys should be made of the normal population from various social and economic levels; (2) attempts should be made to get several specimens (one, if possible following a saline purge) from each person, and arrangements should be made for a portion of each specimen to be placed in PVA fixation immediately after passage, if some hours will elapse before the specimens can be examined; (3) the techniques employed should include a direct or saline preparation, the use of temporary stains for cysts and trophozoites, a concentration technique for cysts, and the use of a haematoxylin stain (the latter time-consuming procedure is the most effective method of differentiating E. histolytica, E. hartmanni, and E. polecki); (4) in reporting the results of a survey, E. histolytica, E. hartmanni, and E. polecki should be listed separately, and under E. histolytica the percentage of patients with amoebic dysentery (trophozoites with ingested blood cells), diarrhoea, and a record of being apparent carriers should be listed; and (5) it should be ascertained that the personnel doing the stool examinations have technical competence.

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The Three Symtoms of Breast Disease

by R. P. Humphreys, 6T2

The newly-initiated clinical neophyte finds statistics tedious. He will be told that breast cancer is the most common cause of death from cancer in the female, with a death rate approaching that for all forms of cancer of the female genital tract combined[1, 2]. He will be told that breast cancer strikes 4% of adult women,[3] and that even with the most effective methods of treatment, only 50% of the patients with the disease will survive five years. When one digests these statistics, one may be confused. Why, for example, should it not be easier to diagnose disease in the breast simply because of its anatomical accessibility to both inspection and palpation, than to diagnose disease in the uterine corpus which is buried deep within the body?

The basic concept of this paper will be to show that breast disease manifests itself as a *pain, lump* or *discharge*. Any one of these suggests alteration in physiology, inflammation or new growth. Thus, in association with a review of developmental history, histology and basic pathology, considerable space will be devoted to the clinical examination of the breast. It will then be hoped that one realizes that all lumps of the breast are not cancer, and all breast cancers are not palpable lumps. One will realize subsequently that the use of sound clinical judgement associated with microscopic examination of suspect tissue makes the diagnosis of breast disease comparatively easy and sure.

Embrology & Histology

From the outset, we must understand the embryological development and histology of the breast for normal tissue structure tends to form "false masses" in the adult female which trap the unwary into believing that these masses are of more serious origin.

In our intra-uterine existence we all developed, about the sixth week, the well-known "milk-line" which ran on the ventral surface between the limb buds. By birth, the "milk-line" had disappeared, and all that remained were two nipples located in the mid-clavicular lines, fifth interspace, bilaterally. From each nipple, approximately fifteen to twenty ectodermal projections penetrated the deeper tissues to form in each case, first the lactiferous duct and then a complex ramification of lobular ducts. (Fig. 1a) The epithelial duct systems were canalized and terminated by a gland bud which would assume an important role later on.

Fibrous and fatty tissue surrounds this duct system, and here and there a condensation of this fibrous stroma courses from the skin through the channel network to the underlying deep fascia. These ligaments of Cooper serve to anchor the breast to the deep fascia, and come into prominence when malignant disease of the breast invades the fibrous tissue and ligaments producing shortening of same, with a dimpling effect being noted on the skin surface.

Thus we have emptying into the nipple, fifteen to twenty lactiferous ducts, each one coming from an autonomous unit (known as a lobe), which units are composed of lobular ducts and gland buds. Up to puberty, the breast is composed mainly of epithelial channels with little supporting stroma.

The male breast remains essentially unchanged throughout life. In the adolescent girl however, the sex hormones take over bringing about an increased amount of stroma and deposition of fat therein, thereby enlarging the breast. With each menstrual cycle the breast tissue ebbs and falls much like the endometrium, estrogen bringing about proliferation of epithelium in the duct systems as well as that in the gland bud. In the latter half of the cycle, progesterone is responsible for stromal growth and edema which creates a sense of fullness in the breast. But as the menstrual period begins, and estrogen and progesterone levels fall, epithelial cells are desquamated, the edema fluid disappears, the stroma atrophies and the epithelial buds and canals regress.

Grossly, the female breast at this stage is variable in size. It normally extends from the sternum to the mid-axillary line covering the area bounded by the second rib above and the sixth rib below. Quite often a finger-like appendage of normal breast tissue extends up towards the axilla, at least as far as the anterior axillary line. This axillary Tail of Spence contains normal tissue which may be mistaken for a tumor arising within the usual confines of the breast. It may actually give rise

to a pathological breast mass which in turn is mistaken for axillary lymphadenopathy.

It is not until pregnancy that the breast adopts its full morphological maturation. From each gland bud numerous grape-like masses project on the ends of tiny ductules which connect with the lobular ducts. (Fig. 1b) These tiny secretory units

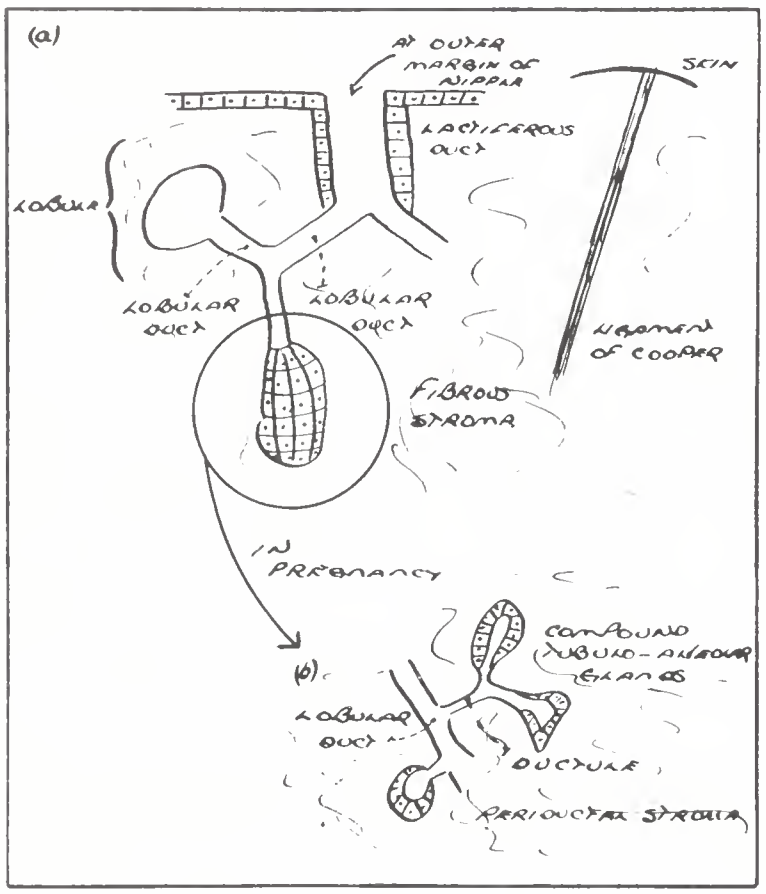

Fig. I. Schematic Diagram of Breast Structure.

become so numerous that they replace much of the supporting stroma. Immediately after parturition, the withdrawal of placental hormone coupled with the action of the hormone prolactin result in the "milk let-down" phenomenom, and later the nursing infant obtains the milk-flow presumably in response to neurogenic or psychic stimuli evoked by him.

After lactation, the glandular structure regresses and the stromal connective tissue once again proliferates. At the menopause, the gland buds, duct system and connective tissue atrophy, until eventually little remains but a few dilated ducts and supporting tissue maintained by estrogen, probably of adrenal origin.

Examination

To the inexperienced, the examination of the breast may be a matter of placing a hand on the organ and glibly pronouncing it free from disease. Sooner or later however, we are all going to be faced by a woman who has a lump in her breast and wishes us to do something about it. This could result in an embarrassing situation if one does not possess an organized plan of attack.

First of all, have the patient in a comfortably warm room, stripped to the waist and seated on the edge of the bed or examining table, in a good light.

Inspection

On inspection, there are three landmarks which meet the eye — nipple, areola and remainder of breast tissue.

Nipple. Normally the nipple points down and out, and occasionally the left is lower than the right. Check for evidence of *discharge* from the nipple. If seen, what is its character? Are the nipples *retracted?* If so, how long has this been the case? If of long standing, the retraction may be cancer, but suggests more often a breast abscess or suppurative necrosis of breast tissue from acute mastitis, with subsequent parenchymal fibrosis and scarring. It may be just simple inversion of the nipple. If the retraction is of recent onset, then it is suggestive of neoplasm with fibrosis proceeding in the breast. Is the nipple (and surrounding areola) involved with *eczema?* Any eczema in this area which lasts longer than two weeks is Paget's disease until proven otherwise.

Areola. On close examination here, remember that *Montgomery's tubercles* like sebaceous glands elsewhere are subject to retention cysts and abscess.

Remainders of Breast Tissue. Compare the two sides for the "4-S", *size, shape, symmetry* and *skin.* With regard to the skin, note if a lump is visible, or if the breast shows obvious signs of inflammation. Is *edema* present? This latter is classically known as "peau d'orange" and can be visualized easier by sqeezing the skin and viewing it through a magnifying glass.

Before leaving this part of the examination, ask the patient to raise her arms above her head. This is a test for tethering or dimpling of the skin which is pathognomonic of carcinoma. A normal breast is mobile in *all* directions over the deep muscles. Raising the arms puts the skin on stretch, and if a mass is present in the breast and is attached to the skin by fibrous bands, the lump is moved and the skin dimples. Dimpling should never be underestimated for often it may point the way to a tumor so small that it would be missed by palpation.

Palpation

The first thing one must do with a woman who has found a lump herself is to ask her to find the lump for you. One observes this site, and then begins palpation on the opposite and supposedly normal breast. This affords one an idea of what is normal for that woman; for many women have irregular areas of breast tissue which feel like ropy bands, in which their length and breadth is greater than their depth. A true mass is equal in all dimensions.

Palpate the breast generally against the chest wall using the flat of the hand (not the fingertips). Then, employing a systematic method of moving radially and clockwise, or from quadrant to quadrant, palpate the tissue between the two hands, one of which is holding the breast away from the body wall. Examine two places specifically, right behind the nipple (and use this opportunity to milk out any discharge), and in the axillary tail. Palpation should be carried out both with the patient sitting and lying down.

If a mass is present, the usual characteristics of a lump are noted as shown in Fig. II. With

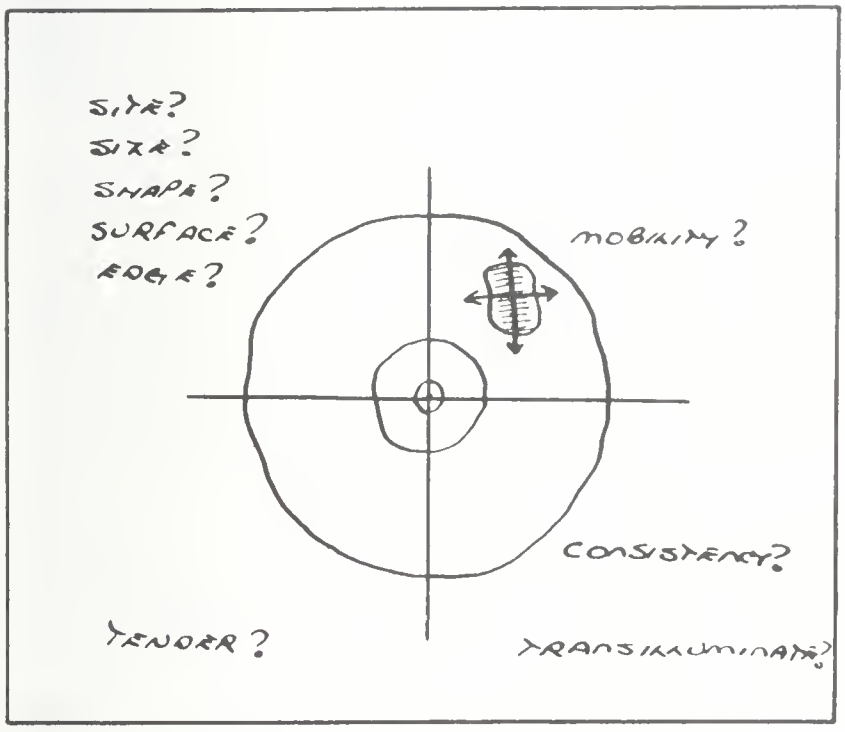

Fig. II. Characteristics of a Breast Lump.

regard to a lump's mobility, one is interested in its relationship to the underlying muscles, usually the pectoralis major. If the latter is put into contraction by pressing the ipsilateral hand into the waist, the mobility of the lump in two planes at right angles to each other is noted and compared with that obtained when the muscle is relaxed. In the instance where the mass lies in the lower outer quadrant, one may have to check mobility over the serratus anterior muscle. This is made to contarct by the patient placing her hand on the examiner's shoulder and pressing, and mobility again checked.

Finally, palpation of the axillary and supraclavicular lymph nodes for lymphadenopathy should be carried out.

Masses in the Breast

Once one feels sufficiently proficient in the examination of the breast, and has performed the inspection and palpation, the next activity is cerebration, that of making a diagnosis. Many of the possibilities of a lump in the breast are included in Table I, but for obvious reasons only the most

TRAUMA		**— Fat Necrosis**
INFECTION	**— ACUTE**	**— Mastitis**
		— Breast Abscess
	— CHRONIC	**— Actinomycosis**
		— Syphilis
		— Tuberculosis
METABOLIC & ENDOCRINE		**— Mammary Dysplasia**
		— Plasma Cell Mastitis
		— Galactocoele
		— Fat Necrosis
NEOPLASTIC	**— BENIGN**	**— Fibroadenoma**
		— Sebaceous Cyst
		— Lipoma
		— Duct Papilloma
		— Chondroma
		— Leiomyoma
	— MALIGNANT	**— Carcinoma**
		— Paget's Disease
		— Cystosarcoma Phyllodes(?)

TABLE I. Differential Diagnosis of Breast Masses According to Etiology

important will be highlighted. Nevertheless, it behooves one who, in the Annual May Games, mentions one of the lesser important masses of the breast, to know something about that mass. This is known as self-preservation!

Table II contains a list of the seven masses in the breast which will be briefly described and itemizes their importance in the various age groups.

Pathologically, the *Fibroadenoma* occurs as a single or multiple, rubbery, mobile mass in the breast, varying in size from that of a pea to grapefruit-size. As the name implies, it is a new growth comprised of fibrous and glandular tissue present in varying proportions. Malignant change is uncommon in the usual small (2-4cms) mass.

Most authors designate a large (about the size of a softball) fibroadenoma as a Cystosarcoma Phyllodes, believing that the latter arises from a

fibroadenoma and is usually benign. However, malignant change can take place, usually from the supporting connective tissue elements, rather than the epithelial channels. Unlike other breast malignancies, blood stream metastases are more common than local lymphatic spread.

	UNDER 35 YRS.	35-50 YRS.	OVER 50 YRS.
FIBROADENOMA	Most common cause	Less common	Rare
MAMMARY DYSPLASIA	Most important cause	Most common cause	Can occur at or after the menopause
CARCINOMA	Can be seen	Second only to mammary dysplasia	Most common cause
MASTITIS	Seen especially in relation to pregnancy and lactation	Less common	Quite rare
PLASMA CELL MASTITIS	Uncommon	Common, especially in parous women	Less common
PAPILLOMA	Uncommon	They must be included, but rarely present as a mass, rather as a discharge.	
PAGET'S DISEASE			Tends to occur in an age group slightly older than cancer

TABLE II. Chronological Division of Important Breast Masses.

Mammary Dysplasia is the most commonly encountered problem involving the breast, yet it is the least understood. It may arise from stromal hyperplasia and duct epithelium proliferation, in which one or the other may predominate, or in which both participate to an equal degree. The latter situation is that which occurs in true "cystic hyperplasia" of the breast.

If one recalls the effects of estrogen on the ductal epithelium, it is not surprising to realize why that hormone has been implicated in the etiology of this disease. It has been postulated that either estrogen is present in absolute excess, or relative excess (where progesterone levels have fallen), or estrogen is operating out of its normal cycle. At any rate, it is believed that the ductal epithelium does not atrophy and desquamate with each menstrual period and there results cystic dilatation of the ducts. At operation the cysts usually exhibit a greenish-yellow translucency, but occasionally a bluish discolouration is noted giving rise to the name "blue-dome cysts".

This is a disease of the reproductive years, for as the breast atrophies post-menopausally, the ductal and stromal hyperplasia regress, but cysts may persist on after the menopause. The clinical features of fibroadenoma and mammary dysplasia are compared with those of carcinoma in Table III.

Carcinoma. One must be awed by the tremendous quantity of material which has been written concerning the etiology, pathology and management of malignant breast disease. Therefore, the following will provide a working clinical structure on which one can build.

Hyperestrinism is thought to cause or maintain mammary carcinoma. Experimental work and statistics show that carcinoma of the breast does not usually develop in the prepubertal period, that prolonged administration of estrogens apparently may lead to cancer, that there is a possibly lowered incidence of the disease in pregnant and lactating females (when estrogen support is depressed), that cancer of the breast is 12 times as common in women between the ages of thirty and forty-nine years who have had mammary dysplasia, and that total oophorectomy may result in a temporary remission of the disease.[5,6] As well, extensive investigation has been carried out regarding a *viral* etiology for breast cancer. Numerous statistics have accumulated regarding the *hereditary* predisposition to the disease.

Pathologically, greater than 90% of breast can-

	FIBROADENOMA	MAMMARY DYSPLASIA	CARCINOMA
INCIDENCE	Commonest benign tumor	Commonest breast problem	Commonest visceral malignancy
AGE RANGE (YRS.)	20-50; esp. less than 25	18-50; esp. 35-50	30 and over
SITE	No preference	Upper outer quadrant	Upper outer quadrant
MULTIPLE?	Either single or multiple	Multiple	Solitary
BORDERS	Very distinct	Medium	Very Indefinite
TENDER?	May be	Yes—especially premenstrually	No
CONSISTENCY	Firm	Vary	Hard
MOBILITY	Slips easily within the fingers to be lost in the deeper breast tissue	Medium	May be fixed
TRANSILLUMINATE?	No	Perhaps	No

TABLE III. Clinical Characteristics of the Three Important Lumps

cers arise from the epithelium of the duct network in the breast. These cancers can be classified into two groups, infiltrating and non-infiltrating. The former group includes scirrhous carcinoma, medullary carcinoma and colloid carcinoma, while the latter group entails papillary and duct carcinoma.

Scirrhous carcinoma is by far the commonest and presents as a hard tumor with cartilaginous consistency which on histological section shows mainly dense collagenous fibrous stroma. Medullary carcinoma is just the opposite in the gross being a large, soft, fleshy tumor which in the microscopic shows irregularly arranged sheets and cords of cells. Colloid carcinoma is also a soft bulky tumor which illustrates generally large lakes of basophilic mucin sometimes in the centre of well-defined glands.

Duct carcinoma begin as a non-invasive anaplastic proliferation of the duct epithelium which grows to the extent that eventually the duct lumen is occluded by neoplastic cells. Often the tumor does not break through the limiting basement membrane, so that no change is perceived in breast tissue. It is only when breast tissue is sectioned that the cord-like ducts packed with neoplastic cells are seen. Papillary carcinoma is the most anaplastic variety of duct papilloma.

Certain local changes may take place in cancer of the breast, such as progression of the tumor to the chest producing fixation, or blocking of the lymphatics producing a local area of edema, (peau d'orange). If the tumor involves the main excretory ducts, retraction of the nipple occurs. Nipple discharge may also be seen. Carcinoma may metastasize via the subareolar and subcuticular lymphatics in a spoke-wheel fashion to the various lymph-nodes shown in Fig. III.7 If the lymphatics are diffusely involved, especially during pregnancy, the breast becomes red, swollen and tender (inflammatory carcinoma).

While the features of these three preceding breast masses have been covered in Table III, it is essential to note that differentiation of a solitary breast mass may be most difficult on clinical grounds. Thus, one cannot afford to gamble one's clinical ability, and must therefore resort to biopsy. This can be carried out as a quick-section with the patient under a general anaethetic. Or, if one suspects that a cyst is present, aspiration of this cyst with a #21 needle (no local anaesthesia required) can be carried out as an office procedure. The diagnosis of mammary dysplasia would be made if: (1) after aspiration the mass has disappeared; (2) the aspirated fluid is clear and straw-coloured and does not contain blood; (3) the mass does not reappear in four to six weeks time. If none of these hold true, or if the mass is solid, quick-section is indicated.

Acute Mastitis is especially common in the nursing mother whose nipples crack and fissure thereby affording entry to bacteria. The commonest organisms are Staphylococcus Aureus and Streptococcus; the former tending to produce localized areas of inflammation and abscess formation, while the latter produces a diffuse inflammation involving the whole organ. The red, hot, swollen area of breast may be accompanied by systemic signs of toxemia. In the early stages of the infection, the affected ducts become plugged with pus, predisposing to suppurative necrosis if left untreated. This necrosis will heal with fibrosis and scar formation with the result that the scar produces a dimpling of the skin, or nipple retraction which may be mistaken for neoplasm at a later date unless an adequate history is taken.

Should a superficial infection extend back along the lymphatics or duct system, a deep-seated breast adscess may result. This is accompanied by heat, redness, tenderness, fever, malaise and pain which is often the most severe of any condition of the breast. Diagnosis can be difficult and one should not wait for the characteristic sign of an abscess (fluctuation) to appear before draining the abscess. Quite often the breast is so hard and tense from the inflammatory response that the abscess may become quite large before it is felt to fluctuate.

Plasma Cell Mastitis tends to occur in the post-lactational breast of a parous woman. Its etiology is obscure but it may be due to a bacterial or viral infection, or may be an aseptic inflammation resulting from inspissation of lipid material within the ducts of the breast. Characteristically the ducts are dilated and contain an inspissated breast secretion which results in an intraductal inflammation. This inflammation may spread to the periductal tissues where it is characterized by plasma cell invasion.

Usually the condition is ushered in with mild pain, tenderness, fever and malaise; but it provides great difficulty in differentiating from carcinoma if instead of the above, a lump develops insidiously and is associated with regional lymphadenopathy.

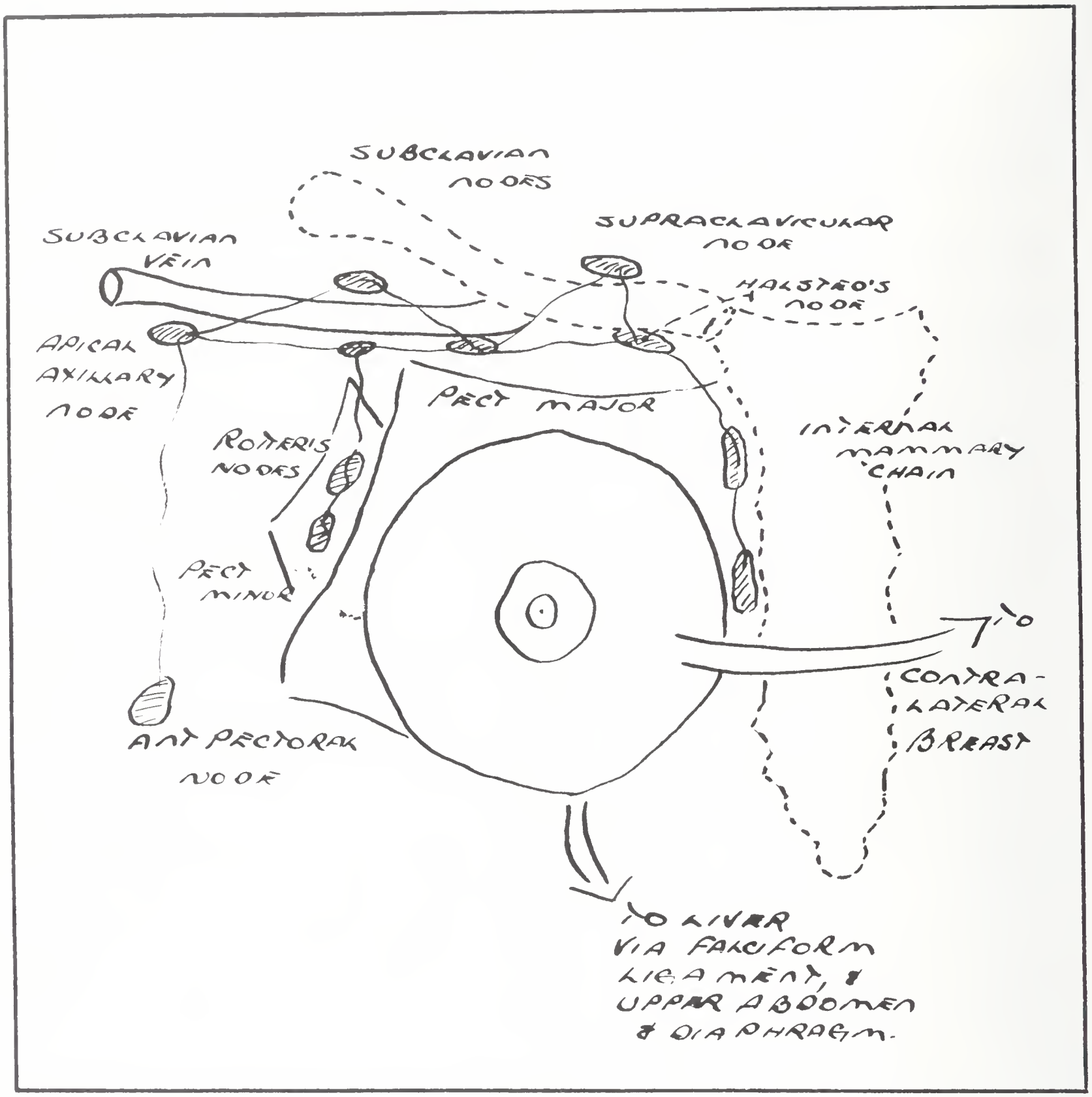

Fig. III. Schematic Diagram Representing Lymphatic Drainage of the Breast

Duct Papilloma are small, usually single, isolated papillary growths occurring in the dilated duct or cyst of an otherwise normal breast. They are made up of a central stromal core which is covered by one or two layers of regular, small, cuboidal cells. These exhibit no evidence of malignancy. While they may present as a breast mass, (due not to the papilloma but rather the dilated duct or cyst), they usually manifest themselves by nipple discharge, this being bloody and serous.

A duct papilloma may occur in several areas and may be associated with mammary dysplasia. In this case the multiple papilloma are called duct papillomatosis, and the histological pattern does not show a regular stromal core and epithelial covering. Many times it is difficult to tell the degree of anaplasia present and whether the lesion is frankly benign or frankly malignant (in the latter case being a papillary carcinoma). No tumor mass is palpable and a bloody nipple discharge is the rule.

Paget's Disease is a variant of intraductal carcinoma with the actual tumor being located in the main excretory ducts. Cells known as "owl's eye" or "Paget's cells" invade overlying epidermis. These cells have a dark nucleus surrounded by a hola of foamy cytoplasm.

The nipple and areola become fissured, ulcerated and ooze. Secondary bacterial infection may complete this eczematoid-like lesion. The importance lies in the fact that any eczema in the region of the nipple which does not respond to conservative management (wet dressings, antibiotics and steroids) in two weeks, should be biopsied.

Pain

Most breast pain (mastodynia) is of non-malignant origin; in fact, a great majority of women experience bilateral breast pain just prior to the monthly menstrual period, as well as in the first, and latter part of the third trimesters of pregnancy. Bilateral pain may also be present in women with mammary dysplasia although it usually predominates in one breast. In females under thirty years of age a tender, discrete lump is most often a fibroadenoma; acute or chronic breast abscesses also produce pain but here the diagnosis is evident from one of the five cardinal principles of inflammation. Pain of recent onset can occur with a malignant mass in the breast, and while one may be guided by the principle of: "Pain before the lump — benign; lump before the pain — malignant", adherence to this temporal relationship is not always the rule, especially in the post-menopausal individual.

Nipple Discharge

This is the last of the triad of complaints which the patient may present with, and may be to her, the most alarming. One should bear in mind that nipple discharge which most often manifests itself as a staining of the brassiere, can arise as oozing from a cracked nipple, or from a denuded area of Paget's disease, or from the lactiferous ducts. The latter could be regarded as true nipple discharge and may be obtainable by milking down successive areas of the breast (like spokes of a wheel). If the specimen obtained by the latter procedure is bright red blood, the origin is probably a benign duct papilloma or duct carcinoma. If it is a clear brown, or yellowish purulent material it is likely to arise from the dilated ducts which occur with mammary dysplasia. If it is milky in colour, then the orgin is physiological lactation (post-partum), and if the discharge is crystal clear one is dealing with a secondary discharge to fibroadenoma or carcinoma.

Nevertheless, all patients, regardless of age, whose spontaneous nipple discharge contains blood, require exploration of the duct.

Psychological Considerations

It goes without saying that the female breast in our own twentieth century society enjoys a rather unique position. Because of certain moralistic taboos it has been transposed to a symbol of sexual attractiveness. We see therefore, in the slick-covered women's magazines, the monthly medical column which deals with breast cancer, squeezed in between those dealing with food, fashion and physical beauty, This hypothetical magazine layout illustrates woman's dilemna.

By self-examination she has discovered a mass in her breast. But all around this central finding come the modifying influences, her physical attractiveness, her husband, her children, her vanity, her socio-professional position and so on. Her thinking is thus divided. One direction will produce fear and ignorance of the mass and perhaps tragic consequences. The other direction will take her to a physician for what may or may not be a happy ending.

As graduating physicians we must realize that this is a problem inherent to our society to which no real blame can be attached. Whether we go

out to become the general practitioner who will in all probability be the first to see a woman with a breast problem, or the general surgeon who may have to remove the breast, we will require a certain amount of savoir-faire in managing the patient, not the disease. Medically and statistically, we have every right to criticize the patient who is afraid to visit her doctor after finding a lump in her breast, but if we do, our callousness and lack of understanding will send our patient elsewhere. And in fact, maybe we ourselves are responsible for the fatalism which these women adopt, believing that the lump is automatically cancer and that they are doomed.

Another realm of our thinking should include the woman who has a mass and who must be operated upon, if only for a quick-section. How do we prepare such a woman pre-operatively? Do we tell her that the mass in her breast can be removed through a small insignificant skin incision within thirty minutes, and that she'll be back in her room in time for lunch? In some cases we may be just that sure of our pre-operative diagnosis, but in others, the very fact that we are performing a quick-section reveals our indecision.

And what do we tell our patient about the radical mastectomy operation if we're sure it must be performed. Do we paint an awful picture? Perhaps in this instance we might be wiser to avoid the histrionic and tell the woman that she should visit a large department store and buy her mastectomy brassiere which she will be able to wear unnoticeably under any article of clothing including a swim-suit. The realization that she can buy such a brassiere off an open counter will help her to believe that she does not by any means have a unique disease.

These are many problems which will not be solved in this article, for like many intangibles in medicine they cannot be spelled out in black and white. Only soul-searching on our part and experience will provide us with a personalized formula, for even the methods which work best for our most respected clinician may be of no value to us because of basic personality differences.

We must therefore, in dealing with problems of the female breast in these 1960's, be well-acquainted with their diagnostic, pathological and statistical importance. But, as well, we must be equally acquainted with our female patient, and her integration to herself, to those who are especially close to her and to the society at large.

ACKNOWLEDGEMENT — My thanks are expressed to Dr. N. A. Watters who encouraged and guided me through this paper.

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UPJOHN ABSTRACT: *A Study of the Psychiatric Effects of Placebo*. Samuels, A. S.; Edisen, C. B. J. Louisiana Med. Soc. 113:114-117 (March) 1961.

Data on the effects of placebo on symptoms in 100 psychiatric patients were gathered in the course of double-blind studies. During placebo administration, 7% of the patients showed marked improvement, 29% improved moderately, 20% improved slightly, 34% showed no change, and 10% became worse. No significant differences in response were seen between the psychotic and the neurotic groups or in the various diagnostic groups. In a study of drug effectiveness, the following percentages of patients would have to show improvement of a given symptom in order to prove that the drug is significantly more effective than placebo alone (providing the plan of the study is comparable to that of the authors' original studies):

affect-flattened	25%	hallucinations	72%
affect-inappropriate	43	sociability-withdrawn	29
association defect	41	insomnia	55
depersonalization	42	anorexia	45
mood-depressed	55	resistance to psychotherapy	26
anxiety	52		
delusions	42		

Unsuccessful attempts were made to correlate response to placebo with patients' characteristics, with characteristics of illness, and with frequency of administration. The only significant correlation was found with race; Negro patients showed more response to placebo than white patients, probably due to differences in transference.

Prospects

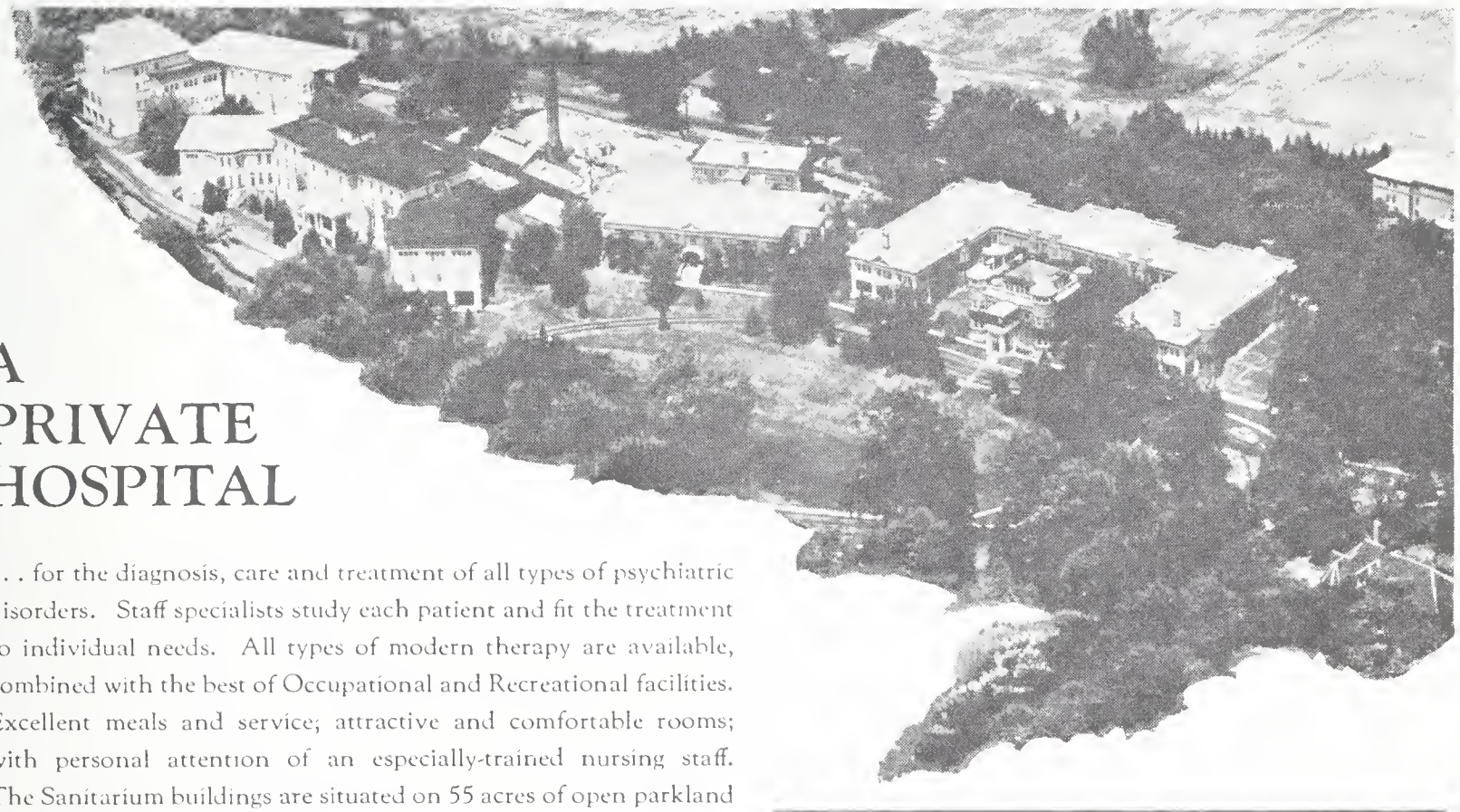
As production of Volume XXXIX of the Medical Journal grinds to a halt, Volume XL is already in the planning stages. The staff has for the most part been chosen, and the topics for presentation roughly determined. Next year, the format of the Journal will be the same: each issue being devoted (in part at least) to a topic of special interest or difficulty to both medical students and practitioners. The five associate editors will each look after one of the first five issues, and it is hoped that the sixth will be a special issue, prepared by a graduate group.

Articles submitted for publication should be less than 3,000 words (12 double-spaced type-written pages) and should be checked by a competent authority. Photographs and drawings are welcomed. The first five topics are, tentatively:

		Assoc. Editor in charge	*Deadline*
Nov. '62	STUDENT RESEARCH	Ruben Baumal	Sept. 17, '62
Dec. '62	BLOOD AND HAEMATOLOGY	John Taylor	Oct. 15, '62
Jan. '63	GENERAL PRACTICE	George McQuibban	Nov. 19, '62
Feb. '63	MENTAL HEALTH	Randy Lee	Dec. 17, '62
Mar. '63	MEDICAL ECONOMICS	Joe Madigan	Jan. 14, '63

A feature which we hope to add next year is a series of reports about medical conditions in countries other than Canada and the U.S. These will take the form of one page (approx. 700 words) articles written by students who visited other countries in a medical capacity over the summer. Photographs are definitely desirable.

With these words we remain silent, until November. J.D.G.



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